The Effect of Gregarine Parasite Infections, Age, and Diet on Calling Song Structure and Mating Behaviour in the Texas Field Cricket, *Gryllus integer*

by

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ABSTRACT

In the past ten years, many researchers have focussed their attention on parasites regarding the role they may play in causing variations in male secondary sexual traits and subsequent effects on female choice. Male age has also been suggested to be an important factor in female choice if old age reflects superior genes. This study investigated the effects that gregarine gut parasites, age, and diet have on the calling and mating behaviour of the male Texas field cricket, *Gryllus integer*.

Male calling songs were recorded in the laboratory using a Digital Signal Processing Network. The song parameters measured were: pulse rate, pulse width, burst duration, pulses per burst, interburst interval, and percent missing pulses. The effects of parasite load and age on the various calling song parameters was investigated in crickets that were fed two different diets varying in nutritional quality. None of the calling song parameters were affected by either parasite load or age in either diet group.

Courtship behaviour was observed and recorded using an Eventlog recorder on an IBM computer in the laboratory. Females mated equally with parasitized and unparasitized males and with old and young males The total duration and proportion of time spent performing each of 9 courtship displays were recorded for males on each diet. Only one display was affected by parasite load. Highly parasitized males fed the nutritionally inferior diet juddered for a proportionately shorter time than males with low parasite loads. Also, older males performed juddering and shaking antennae proportionally

longer and juddering and raising wings for longer durations than younger males.

Males that successfully mated were observed for performance of 8 post-copulatory guarding behaviour displays. None of the guarding behaviours were affected by parasite load. However, one display was affected by age, with older males performing guard turning for shorter durations than younger males. Results are discussed in terms of the influence of parasites and age on female choice.

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INTRODUCTION

Darwin (1871) proposed that sexual selection, through female choice, could explain the evolution of the elaborate male secondary sexual traits seen in many animal species. These traits and ornaments may be representative of male genetic quality. Therefore, variation in the male characters or behaviours might reflect heritable genetic variation (Zahavi 1975, 1977). Females who choose males based on differences in these secondary sexual traits might gain heritable benefits for their offspring (Trivers 1972).

Two factors which have been proposed as variables important in female choice are male parasite resistance and male age. Hamilton and Zuk (1982) proposed that females assess the elaborate displays of males to determine the degree of parasitism of the male. Males who are resistant to parasites should have traits and displays which are more vigourous and elaborate than males who are parasitized. Females may also choose to mate with males who are older since they have proven their ability to survive and therefore carry superior fitness genes (Manning 1985). Old males, with a decreased residual reproductive value (Stearns 1992), should increase their reproductive effort and display traits more vigourously. If variation in male displays are attributed to parasites or age, females could assess these differences and choose mates who are the most vigourous and possess the most superior genes.

Field crickets (Orthoptera: Gryllidae) represent a species where several male mating displays can be measured, and the effect of parasite load and age can be assessed. Previous studies have shown that gregarine gut parasites may decrease some components of fitness

in crickets (Zuk 1987 a, b) and that age may influence mating success (Zuk 1987 c, Simmons and Zuk 1992). The purpose of this study was to determine if variations in the calling song structure, courtship, and post-copulatory mate guarding behaviour of the Texas field cricket *Gryllus integer*, could be attributed to differences in gregarine gut parasite loads and age.

LITERATURE REVIEW

In this section, literature that is relevant to the thesis will be reviewed. The areas focused on are: natural and sexual selection, female choice, parasites and female choice, age, and Orthopteran mating behaviour. An introduction is given at the beginning of each section outlining the topics which are discussed.

NATURAL AND SEXUAL SELECTION

Darwin (1871) was the first to formally developed a theory of evolution through natural selection. His theory of evolution was based on the central theme of "the struggle for existence" or "the survival of the fittest". Natural selection is the differential survival of individuals who are able to acquire more resources and are better adapted to their environments to survive and reproduce.

As a subset of his theory of natural selection, Darwin also proposed the theory of sexual selection. Darwin was led to the idea of sexual selection because of the problem posed by such traits as the tails of peacocks or the antlers of male deer. These traits do not contribute to survival of the individual or to their offspring, but may confer advantages to certain individuals over other individuals of the same sex and species in relation to reproduction (Darwin 1871). Sexual selection depends not on the struggle for existence, but on competition between individuals of one sex to mate with individuals of the opposite sex and the advantage some individuals have over others in attracting mates. The result of such struggles is not death to the unsuccessful competitor, but fewer or no offspring.

There are two components of sexual selection: competition for mates between members of the same sex (usually the males) and the

active choice by one sex for particular individuals of the other sex (usually females choosing males). In many species, the reproductive investment of males is relatively cheap since sperm production is inexpensive whereas the investment is much greater for females as egg production is energetically costly. Due to this disparity in investment in gamete production between the sexes, females are usually the choosy sex while males compete aggressively for mating opportunities (Trivers 1972). However, Darwin did not propose a mechanism for how female choice may evolve. It wasn't until almost fifty years after Darwin (1871) first proposed his theory of sexual selection that other researchers attempted to explain how female choice may have evolved (Maynard Smith 1985).

EVOLUTION OF FEMALE CHOICE

Several mechanisms have been proposed to explain the evolution of female choice. Females may choose males to gain resources that will directly benefit them and their progeny or they may choose their mates based on traits that will indirectly increase the viability of their offspring. This section outlines some of the most common theories proposed to explain the evolution of female choice.

Resource Based Female Choice

One mechanism by which female choice may evolve was based on observations that some females tend to mate with males who possess superior resources. This is termed resource based or direct female choice. Selection will favour females who choose to mate with males who offer superior resources for the female and her offspring (Kirkpatrick 1985). Males of many polgynous animal species contribute nest sites, nutrition, parental care, or other material benefits to their mates in addition to their gametes. Male bullfrogs, *Rana calesbeiana*,

defend oviposition sites and those males who defend sites which are of ideal temperature and contain few predators are chosen more often by females (Howard 1978). Price (1984) found that female Darwin's finches choose to mate with males that possess territories which contain superior food resources. Female katydids, *Requena verticalis*, select males who provide them with superior nuptial feeding gifts (Gwynne 1988).

Non-Resource Based Female Choice

Non-resource based female choice occurs when choice does not necessarily give the female direct benefits such as food or territory (Fisher 1930). Several theories have been proposed to explain how female choice could evolve for male traits which do not directly benefit the female or her offspring. Three of these non-resource based theories are described below.

Fisherian Female Choice

A mechanism for the evolution of female choice based on traits which do not directly benefit the female was proposed by Fisher (1930). He suggested that a novel genetic male character spreading through a population as a consequence of a slight natural selective advantage, might become the object of a genetically determined female mating preference. Females exercising the preference would produce fitter progeny because they have paired with males that have a genetically controlled natural selective advantage. Those males which possess the trait picked out by females will gain a reproductive advantage because they will be more likely to be chosen by females than males lacking the preferred character. Both the female genes for the trait preference and the male genes causing the trait will be passed on to the progeny. The female preference and the preferred character will consequently

increase in frequency and reinforce each other. Unless checked by some other counter selection, the rate of in-step coevolution will increase geometrically giving rise to what has been termed "Fisher's runaway process of sexual selection" (Andersson 1982 a). For example, in a population of widowbirds (*Euplectes progne*), some females may prefer to mate with males bearing an extravagant mating character such as a longer tails. Therefore, males with longer tails do better by securing more mates (Andersson 1982 b). Choosy females with more intense preferences also do better because their sons are more likely to have long tails and, like their fathers, be more attractive and have a reproductive advantage. So choosy females get more grandchildren through the success of their sons. However, such exaggeration of a trait will reach a point where natural selection will check the runaway process and limit the tail length when it hinders the ability of the male to obtain resources and remain competitive.

Through population genetic modeling, O'Donald (1980), Lande (1981), and Kirkpatrick (1982) have supported Fisher's process of sexual selection. Both Lande and Kirkpatrick, however, claim that their models of sexual selection show that, unlike Fisher's model, no initial selective advantage for female preference is necessary for the origin or subsequent elaboration of male mating traits.

Sensory Exploitation

Another theory to explain the evolution of female choice was proposed by Andersson (1990) and Ryan *et al.* (1990). The sensory exploitation hypothesis explains male secondary sexual characters as adaptations to exploit female responses that evolved in non-sexual contexts. Unlike other non-resource based female choice models, the sensory exploitation model does not require a process where the male

trait and female preference co-evolve to explain the evolution of elaborate male secondary sexual characters. The sensory adaptation model involves exploitation of a previously developed female sense by novel male characters. The sense in the female evolved for a purely non-sexual reason and is exploited by the males in a sexual context.

Evidence to support this theory comes from a study performed by Proctor (1992). Female water mites, *Neumania papillator*, have evolved to recognize prey vibrations and will orient towards the source of the vibrations. Males exploit this response of the females by performing courtship trembling behaviour at a frequency similar to that of moving prey. It is assumed that the female response had evolved for the non-sexual purpose of prey capture and the male courtship trait evolved later for attracting females.

Good Genes Models

A female may gain indirect benefits for her offspring by mating with a male of higher genetic quality. In species where males give no paternal investment to mates or their offspring except the donation of sperm, any direct benefits to the female are absent. Yet many males in such systems have elaborate secondary sexual ornamentations. Often these species have exaggerated sexual dimorphism in secondary sexual characters. This suggests that where paternal investment is absent, and female choice is costly, females may be selecting males in order to gain superior genes.

There are certain risks associated with mate choice. Animals may increase their risk of predation when seeking a mate, and when in courtship or in copula. This is certainly the case for males which produce highly conspicuous mating signals to attract females (Andersson 1982 b). If female mate choice occurs through the

attraction and surveying of many males, or through multiple courtships and copulations, females that are more choosy often suffer higher levels of predation. Courtship and copulation may take between a few seconds and several hours, during which other activities are precluded (Thornhill and Alcock 1983). In addition to time and predation costs, choosy females may incur greater energetic costs and higher probabilities of injury, and increase the chances of obtaining a disease (Pomiankowski 1987). Female preferences may have direct effects on female survival and fertility.

The offspring of choosy females not only have a Fisherian reproductive advantage but also have greater viability. This suggests that in species with exaggerated male ornamentation in which female choice is costly, it is likely that female mate choice will be for traits that correlate with male genetic quality (Andersson 1982 b). Exaggerated male characters act as markers or revealers of the genetic quality of potential mates. If females choose their mates using traits that correlate with heritable viability differences, then stable exaggeration of both female choice and the preferred male character is possible even when female choice is costly.

Since it is costly for females to produce hybrids, there is selection on females to choose males with characters distinctive of their own species (interspecific). Females also choose between males of the same species (intraspecific) for their ability to survive to old age, overall vigour, male dominance in aggressive encounters, and disease resistance.

Although choosy females often incur greater costs associated with mate choice, their increased reproductive success through choosing better quality males may offset these costs (Majerus 1986). Females

who exhibit mate choice should have increased reproductive success. In a study by Partridge (1980), female *Drosophila* who were able to choose their mates had higher reproductive success and more fit offspring than females who were assigned mates. Similarly, Crocker and Day (1987) found that female seaweed flies, *Coelopa frigida*, had more offspring survive when they had choice of mates compared to females who had no choice. Reynolds and Gross (1992) found that female guppies, *Poecilia reticulata*, choose to mate with larger males which resulted in offspring with higher growth rates.

Handicap Principle

An aspect of the "good genes model" is the Handicap Principle. This widely discussed hypothesis of female choice for male genetic quality was first proposed by Zahavi (1975, 1977). A handicap is defined as a male secondary sexual character that reduces male survival chances and in addition acts as a mating signal. The theory predicts that female preferences for handicapping ornamental traits are established since these traits act as markers of male health and vigour, with only the healthiest males are able to produce the ornament and survive to exhibit the handicap.

Maynard Smith (1985) classified models of the "handicap principle" into three distinct types: "Zahavi's handicap", the "condition-dependent handicap", and Hamilton and Zuk's "revealing handicap" (1982). These models all suggest that it is possible for females to choose mates of higher viability and fitness by assessing a male's secondary sexual ornaments.

Zahavi's Handicap

Zahavi (1975) made the claim that male ornamental characters, which handicap male survival, have evolved because of their role as

markers of male genetic quality. A handicapping ornament acts as a viability marker because only males with high general viability can survive the burden of bearing an ornament. Females that prefer to mate with handicapped males are more likely to have offspring with higher than average viability.

Female mate choice for male genetic quality can only be based on a system that guards against cheating (Zahavi 1975, 1977). The reliability of an advertisement can only be assured if the handicap is costly to produce. For a trait to act as Zahavi's handicap, males with the handicap must survive relatively well when they have high viability but poorly when they have low viability. This implies that viability interactions between genes for the handicap and genes for viability must be non-multiplicative, that is, the high viability gene must be at a higher frequency in handicapped males than in non-handicapped males.

However, there are many problems with this model. Females who prefer males with costly ornaments may increase the probability of their offspring inheriting genes for high viability but they also burden their offspring with genes for the handicap (Maynard Smith 1976). Zahavi claimed that if female choice allows females to mate with mates that have higher heritable viability then the loss to female personal fitness through a costly choice could be offset by the increase in her inclusive fitness through bearing high viability offspring (Zahavi 1975). The key dilemma for Zahavi's handicap is that the reliability of the ornament as an indicator of fitness can only be achieved if the ornament is highly deleterious which generates very strong natural selection against the ornament. Also, if a viability allele is highly favoured, the viability allele will go to fixation in only a few generations

after which any advantage is lost (Davis and O'Donald 1976). These problems, however, are resolved in the following two handicap theories.

Condition Dependent Handicap

More recently, it has been theorized that there may be condition-dependent handicaps which are mating ornaments that are expressed in proportion to the phenotypic condition of its bearer (West Eberhard 1979, Andersson 1986). Only the males with the high viability allele and the handicap gene can produce the mating ornament. Low viability males with the handicap gene are assumed to be unable to make the ornament. Therefore, the low viability males with the handicap gene are indistinguishable in appearance from the true non-ornamented males that lack the ornament gene. Net viability selection against the handicap gene is reduced as only those high viability males best able to make the mating handicap do so. The ornament acts as a very reliable marker of high genetic quality. Choosy females that prefer handicapped males without fail will mate with males that have the high viability allele. These two effects act on mate choice by decreasing the costs of producing handicapped sons and increasing the benefits by producing high viability offspring.

Hamilton and Zuk Hypothesis: Revealing Handicap

Courtship in birds can be used as an example to distinguish between the condition-dependent handicap and the revealing handicap. Courtship often involves elaborate dance movements, vocalizations, and the display of feathers and crests. When weak or sick, individuals cannot perform the courtship or simply stay away from the display area. The courtship itself, and not the ornament, can be considered to be a condition-dependent handicap, since low viability males completely avoid the energetic costs and predation risks of courtship.

However, in contrast to this, if the courtship of weak or sick individuals is of poorer quality than that of strong or healthy individuals, or if the weakness is revealed in the courtship displays, then the courtship can be considered to be a revealing handicap. In this case, the low viability individuals still incur the costs of display and in building the ornament in the first place.

Hamilton and Zuk (1982) have related this idea to their host-parasite model by assuming that females are able to distinguish the degree of past or present parasitism of a male from the condition of the male's ornament(s). All males with the handicap gene produce an ornamental phenotype, but the condition of the ornament in a male with high viability is noticeably different from the condition of the ornament in a male with low viability. These noticeable differences in viability make healthy males more attractive and unhealthy males less attractive. Hamilton and Zuk noted that parasites are ubiquitous and suggested that continuous coevolution between parasites and their hosts might drive the evolution of preferences for extreme male displays. They postulated a correlation between the genes for resistance to parasites and the expression of the male trait. A variety of mechanisms could produce this correlation, the simplest being that more resistant males will be healthier and in better condition to grow elaborate plumage and perform strenuous displays. Then females with preferences for the most extreme males will also tend to mate with those males that are the most resistant to disease. This establishes a genetic correlation between the preference and resistance genes so that the evolution of greater parasite resistance also causes the evolution of more extreme preferences. The male trait will become exaggerated as a result.

The critical assumption that keeps the trait and preference from reaching an equilibrium, as it might in Zahavi's model, is that the genes responsible for resistance are assumed to be constantly changing. If females choose males based on good health and vigour, a continuing source of heritable genetic variation in fitness must be present in the population (Falconer 1981). Parasites can provide fitness variation because they evolve rapidly and dynamically with the host (Hamilton 1982).

Hamilton and Zuk (1982) proposed that populations may evolve cyclically in response to parasites. The process will be cyclical because new resistant genotypes that arise in the host species will provoke the evolution of new parasite genotypes and these will in turn provoke the evolution of new host genotypes. This explains how a population can have substantial additive heritability for viability and disease resistance. Females may be able to detect whether or not males have high parasite loads, but only if the males also have handicapping ornaments. A male that is relatively free of parasites would develop the trait to a greater degree than a heavily parasitized male. It would be advantageous for a female to select a male with a handicap, indicating that he has fewer parasites.

In order for the Hamilton and Zuk hypothesis to work, female preference must emphasize those signs of male health that are true indicators of male viability because they are energetically costly to produce (Pomiankowski 1987). The hypothesis predicts that across species, parasite prevalence should correlate positively with male brightness, with brighter species having more parasites. The hypothesis also predicts that within a species, parasite loads should correlate negatively with mating success of individual males. The parasite

hypothesis is based on three assumptions: 1) the full expression of secondary sexual characters depends on the health and vigour of the individual possessing them; 2) some species are continuously coevolving with parasites, so that host resistance to parasites remains heritable; 3) some of these parasites have appreciable effects on viability. If these assumptions are true, female choice should be based on the condition of potential mates' secondary sexual characters if it will increase offspring fitness (Møller 1990 c).

PARASITES AND FEMALE CHOICE: EMPIRICAL STUDIES

The Hamilton and Zuk (1982) model has two main predictions: that interspecifically, those species which are host to the most parasites should have the best developed secondary sexual traits and intraspecifically, individuals that are resistant to parasites should have the most developed secondary sexual traits. This section will review literature pertaining to the influence of parasites on female choice that test both predictions in a variety of animal species.

Interspecific Tests

The original Hamilton and Zuk hypothesis (1982) was based on an interspecific study of 109 species of North American passerine birds. The study was conducted to test the interspecific part of the hypothesis; those species with the greatest number and diversity of parasites would have the brightest feather plumage and the most elaborate songs. The brightness of male plumage and complexity of male songs were scored for various species of North American birds and compared to the number of blood parasites associated with each species. A positive association was found between parasite prevalence and both male brightness and the complexity of male song. This supported the

prediction of the hypothesis: male brightness and song complexity correlated positively with parasite load across species.

The methods of this study, however, were criticized for ignoring the possible effects of ecological variables such as nest height, diet, and flock size (Borgia and Collis 1989). It also failed to take into account the problem of taxonomic non-independence of the North American passerine birds. Read (1987) re-examined the data from the Hamilton and Zuk study and, in addition, examined a data set on European passerine birds taking ecological variables as well as taxonomic effects into account. He found that the correlation was actually strengthened by these refinements. Yet, later re-analysis by Read and Harvey (1989) did not support either Hamilton and Zuk's (1982) or Read's (1987) initial findings. In this later study, the brightness of North American and European birds were re-scored. Using the new scores, they found no correlation between male brightness and parasite prevalence in the North American birds. For the European passerines, a weak possitive relationship was found, but this was due only to phylogenetic associations.

Read and Weary (1990) re-tested the song data from the Hamilton and Zuk (1982) study. Hamilton and Zuk had found that species with higher haemotozoa prevalence tended to have more variable and complex songs. However, when Read and Weary used quantitative data on more specific parameters of song, they found a significant negative relationship between parasite prevalence and song continuity contrary to the direction predicted by Hamilton and Zuk. The positive correlations came about through taxonomic associations only. Within taxa, there were no relationships found between any of the song variables and parasite prevalence.

Several studies have been conducted on other closely related species to test the interspecific prediction of the hypothesis. Weatherhead *et al.* (1991) investigated the effect of a haemotozoan blood parasite on ten species of wood-warblers (Parulinae). Plumage dimorphism and plumage brightness were measured. No evidence was found that haematozoa prevalence was correlated with either male plumage brightness or plumage sexual dimorphism in the 10 species of warblers. To explain why their study did not support the Hamilton and Zuk hypothesis, Weatherhead *et al.* (1991) suggested that there are problems with quantifying plumage brightness in birds since there is substantial variation in the assessment of brightness by different observers. Measuring plumage brightness is very subjective and gives no indication of what female warblers perceive as bright plumage. Also, it is often difficult to estimate parasite prevalence since parasite intensity is highly variable.

A study of both endoparasites and ectoparasites of New Guinea birds using data specifically collected to test the Hamilton and Zuk hypothesis was conducted by Pruett-Jones *et al.* (1990). This study controlled for phylogenetic effects, diet, foraging height, sample size, and used several different measurements of parasite load as well as "brightness" and "showiness" measures. They found that most of the predicted positive relationships between parasites and sexually selected traits remained, with the species that were host to the most parasites being the brightest and showiest.

Interspecific tests of the Hamilton and Zuk hypothesis have not been limited to birds. The relationships between parasitism, sexual dichromatism, and life history variables in six species of British and Irish freshwater fish were investigated by Ward (1988). The analysis

strongly supported the interspecific hypothesis proposed by Hamilton and Zuk as the species with higher parasite loads were more sexually showy.

Zuk (1991) tested the hypothesis but designed the study to explain why some species have more parasites than others. Most other host-parasite studies give no explaination why some species have more parasites than others. Zuk surveyed Neotropical birds and subdivided them into migrant and resident species. The prediction was that resident species, because of their greater capacity for forming close coadaptational relationships with parasites, should show the relationship between parasites and brightness more strongly than species that migrate. This prediction was upheld in the study.

Intraspecific Tests

Most studies that test the Hamilton and Zuk hypothesis test the intraspecific prediction. This proposes that individuals within a species that have few parasites, and therefore high parasite resistance, will be more showy and be preferred by females of that species. Table 1 gives a summary of results from some intraspecific tests of the Hamilton and Zuk (1982) hypothesis on various animal species. The following section reviews several intraspecific studies and the effect of parasites on the overall health and vigour, secondary sexual characters, mating displays, and mating success of several animal species.

Barn swallows (*Hirundo rustica*) are parasitized by haematophagous mites (*Ornithonyssus bursa*) that suck the blood of their hosts. Mite infections have been shown to reduce male viability, decrease adult size and weight, and reduce mating success (Møller 1990 a, b). An important secondary sexual trait in female choice, tail length, is also affected by parasite levels. It was found that males with fewer

Table 1. Results from some intraspecific tests of the Hamilton and Zuk (1982) hypothesis.

Assuptions and Predictions	Field Crickets	Fruit Flies	Damsel-flies	Guppies	Gray tree Frogs	Pheasants	Jungle Fowl	Sage Grouse	Rock Doves	Barn Swallows	Satin Bowerbirds	Red-winged Blackbirds	Mice	Deer
Expression of ornaments dependent on parasite load	Y/N	--	--	Y	N	Y	Y	Y/N	?	Y	N	N	--	Y
Resistance is heritable	--	--	--	Y(?)	--	Y	Y	--	--	Y	Y	--	--	--
Negative effects of parasites on host fitness	Y	Y	Y	Y	--	Y	Y	--	?	Y	Y	N	--	Y
Females choose males with fewer parasites	N	--	--	Y	N	Y	Y	Y	?	Y	Y	--	Y	--
references:	1, 2	3	4	5, 6, 7	8	9	10	11, 12	13	14	15	16	17	18

Yes (Y), No (N), Yes and No (Y/N - supported by some papers but not by others)

* References: 1. Zuk (1987 a, b, c, 1988), 2. Simmons (1990), 3. Jaenike (1988), 4. Forbes and Baker (1990), 5. Kennedy et al. (1990), 6. McMinn (1990), 7. Houde and Torio (1992), 8. Hausfater et al. (1990), 9. Hillgarth (1990), 10. Zuk et al. (1990 a, b), 11. Boyce (1990), 12. Gibson (1990), 13. Clayton (1990), 14. Moller (1990 a, b), 15. Borgia and Collis (1989), 16. Weatherhead et al. (1993), 17. Kavaliers and Calwell (1992), 18. Mulvey and Aho (1993).

parasites have longer tails (Møller 1990 b). Data has shown that female barn swallows avoid mating with heavily infected males (Møller 1990 a) and cross fostering studies suggest resistance to these parasites has a genetic basis (Møller 1990 b). Song output, used by males to attact females and to repel conspecific males, was found to be reduced in males with high levels of mite parasite infections (Møller 1991).

A variety of other studies on birds have supported various aspects of the Hamilton and Zuk hypothesis. As in barn swallows, male black grouse (*Tetrao tetrix*) who have fewer blood parasites (*Leucocytoozoon lovati* and *Splendidofilaria tuvensis*) also have longer tails (Hoglund *et al.* 1992). Hillgarth (1990) found that ring-necked pheasant females (*Phasianus colchicus*) prefer to solicit copulations from males that are naturally resistant to several species of *Eimeria* coccidian parasites. Also, it was found that resistance is heritable in natural populations since chicks from naturally resistant fathers survived parasite infections better than chicks from fathers that were experimentally cleared of parasites. Boyce (1990) found in sage grouse (*Centrocercus urophasianus*) that lice parasite loads (*Lagopoecus gibsone* or *Goniodes centrocerci*) and strutting frequency were correlated. Parasite free males had longer durations of strutting frequency which is a secondary sexual character of males.

Several observational and experimental studies on female choice for parasite-free males have been conducted. After experimental manipulation of lice loads (*Columbicola columbae*) in rock doves, *Columba livia*, Clayton (1990) found that females preferred males free of parasitic lice. Clean males displayed more often than lousy males who were subject to plumage damage. Mites (*Myrsidea ptilonorhynchi*) were also implicated in mate choice in satin bowerbirds,

Ptilonorhynchus violaceus. Males with fewer parasites were better able to hold a bower, and therefore secure access to more females (Borgia and Collis 1989). The relationship between parasite load and secondary sexual characters was also studied in captive red jungle fowl, *Gallus gallus* (Zuk *et al.* 1990 a). Females preferred males with long, brilliantly coloured combs, redder eyes, redder feathers, and longer tail feathers. Eye colour, and the colour, texture, and size of the comb have been correlated with intestinal roundworm parasite infections, with parasitized males exhibiting duller and inferior traits. Therefore, these characters may be indicators of the current state of a male's health and well-being (Zuk *et al.* 1990 b).

Although the studies on birds mentioned above have supported various parts of the Hamilton and Zuk hypothesis, there have also been many studies where parasites seem to have little or no effect on male traits and female choice. Weatherhead and Bennett (1992) studied the effects of haematozoan parasites on brown-headed cowbirds, *Molothrus ater*, and ecto- and endoparasites in red-winged blackbirds, *Agelaius phoeniceus* (Weatherhead *et al.* 1993). Both studies failed to find any correlations between parasite loads and several male secondary sexual traits (e.g. feather condition, body size, epaulet size, colour, song repertoire) or viability. Also, infections of a genus of haematozoan parasites, *Haemoproteus*, did not affect male courtship displays or mating success in sage grouse, *Centrocercus urophasianus* (Gibson 1990).

Birds, because of their colourful plumage and elaborate courtship displays, are most often the group of animals examined with respect to testing the predictions and assumptions of the Hamilton and Zuk hypothesis. However, several other species have also been used to test

the hypothesis. For example, several species of fish have complex displays which are part of their mating behaviour. There is evidence that nematode parasites (*Camallanus cotti*) affect courtship displays and female choice in guppies, *Poecilia reticulata* (McMinn 1990, Kennedy *et al.* 1987). The rate of courtship behaviour was reduced by parasite infections. Also, Houde and Torio (1992) found that male guppies infected with external monogenean parasites (*Gyrodactylus turnbulli*), were dull in colour and females spent less time near such dull infected males and did not respond to their courtship displays. However, Ward (1988), in the study on British and Irish freshwater fish, found no significant correlation between mean parasite abundance per individual and the degree of sexual dichromatism, although this may have been due to the small sample sizes.

Several insect species have been studied to determine the impact of parasite infections on fitness. Water mite (*Arrenurus* spp.) infections on adult damselflies, *Enallagma ebrium*, negatively affected the mass and condition of males (Forbes and Baker 1990). The effect of a parasitic nematode, *Howardula aoronymphium*, on the male mating success of *Drosophila testacea* was investigated by Jaenike (1988). Parasitized males had lower mating success than unparasitized males.

Orthopteran insects have also been used to test the hypothesis. Simmons (1990) studied post-copulatory guarding, female choice, and levels of gregarine gut infections in the field cricket, *Gryllus bimaculatus*. Heavily infected males spent longer actively guarding females than lightly infected males suggesting that heavily infected males had to invest much more energy to keep a female close. Lightly infected males were able to guard the female more successfully than

heavily infected males since females remained close to these males longer after mating. *G. pennsylvanicus* field crickets infected with gregarine parasites suffered reduced longevity, greater weight loss, and reduced spermatophore production relative to uninfected males. Also, the developmental period of infected males was significantly longer compared to uninfected males (Zuk 1987 a).

Apart from birds, fish, and insects, only a few other species of animals have been investigated. Hausfater *et al.* (1990) found that helminth parasite loads (*Dorylaimus* spp.) in gray treefrogs, *Hyla versicolor*, were not correlated to growth or development and did not affect male calling song. Similarly, no correlation was found between mating success and levels of blood-feeding parasites, *Pseudodiplorchis americanus*, in spadefoot toads, *Scaphiopus couchii* (Tinsley 1990).

Mulvey and Aho (1993) studied liver flukes (*Fascioloides magna*) in white tailed deer, *Odocoileus virginianus*. Parasite infections were associated with lower weight and a reduced number of antler points in young males. Kavaliers and Colwell (1992) investigated mate choice in female laboratory mice (RML, Rocky Mountain Laboratories, Hamilton, MT) who were exposed to the scent of males infected with a protozoan parasite, *Eimeria vermiformis*. Female mice preferentially choose the odour of unparasitized males.

Problems With Testing the Hypothesis

The evidence from the various intraspecific studies that test the Hamilton and Zuk hypothesis are equivocal in their findings. While some studies lend support to the hypothesis, others found no relationship between female choice and male parasite load. A few researchers have suggested reasons to explain the ambiguity of these results.

The results from Weatherhead and Bennett (1992) and Weatherhead *et al.* (1991) indicated some critical problems with using haematozoa infections in birds to test the Hamilton and Zuk hypothesis. They found significant variation in overall parasite prevalence within and between years, temporal differences in the prevalence of parasites, and significant seasonal variation in the intensity of infections within and between years. They suggest that if parasite prevalence is so variable, these particular parasites are probably not a reliable cue to assess resistance. Therefore, haematozoa parasites may not be appropriate parasites for testing the hypothesis and this type of parasite infection may only become detrimental to the host under stressful conditions.

Since many animals are hosts to a variety of parasites, the parasite that is chosen for study in a particular investigation may not always be the one with the greatest effect on male health. Another parasite that is not being measured may actually have a much greater effect on male viability. Weatherhead *et al.* (1993) noted that in several red-winged blackbird studies, failure to find a correlation between a particular parasite and a particular secondary sexual trait could simply mean that the wrong parasite or the wrong trait was studied. Gibson's study (1990) on sage grouse also suggested that other parasites might be involved to explain individual variation in display and mating success and the effects of parasites may only be one of several mutually interchangeable processes maintaining the variation.

Hoglund *et al.* (1992) noted that females may avoid infected males simply to avoid exposure to, and possible transmission of, the parasite. Rather that choosing parasite-free mates to acquire genetic resistance, choosers may select them simply to avoid the direct transmission of

parasites to themselves or their offspring (Borgia and Collis 1989). Also, it may be that higher parasite loads are indicators of general stress rather than genetic resistance. Females may therefore mate with more vigourous males for reasons unrelated to parasite resistance.

Pruett-Jones *et al.* (1990) found that in birds of paradise, *Parotia lawesii*, the variable most affected by parasites (*Leucocytozoan* spp. and *Trypanosoma* spp.) was court attendance. Females may be avoiding parasitized males simply because they are not at the courtship site and not because the are actively choosing resistant males. If females simply avoid parasitized males in any given year, all males with low intensities could mate, regardless of whether these males have low intensities every year or only in the year they mate. In contrast, if females actively choose resistant males, and long-term resistance is perceptible to females through some characteristic of males, only those males with consistently low infections should mate in any given year.

In some species, selection may be stronger for qualities other than parasite resistance. Tinsley (1990) stressed that in spadefoot toads, individuals are subject to intense selection pressure: high mortality at the tadpole and juvenile stages, a short feeding season with intense competition, prolonged hibernation, and parasite infection. Hosts which survive hibernation in poor condition are selected against, but not by mate choice. They simply never make it to the spawning assemblies. This study suggested that selection for disease resistance may occur at a far earlier stage than at the mating arena. Early selection for disease resistance may be a very common phenomenon in many species where selection is strong and there is a high rate of juvenille mortality.

AGE AND FEMALE CHOICE

Females may assess male age during mate choice. Age may be an accurate indicator of fitness, with older males having a higher average fitness than younger males (Manning 1985). Females should choose to mate with older males since they have demonstrated their capacity to survive. Therefore, females who choose older mates produce offspring which have higher fitness than progeny from randomly mating females (Weatherhead 1984). It has been shown that the size and complexity of many male ornaments and weapons are positively correlated with age. As female choice genes for age spread, the relationship between the age-dependent ornament and choice for it increases and also spreads.

Females may prefer characters which are correlated with age which also enable males to increase their reproductive success. For example, larger *Anolis garmani* lizards obtain more copulations than small males and male size is closely correlated with age (Trivers 1972). Although many mammals and birds reach a definitive size, maturity is usually reached before growth stops so that male size has some correlation with age. Long *et al.* (1980) found that older *D. melanogaster* males were more successful in mate tests than young males.

In addition to ornaments and weapons, age dependent female choice may be directed towards male behavioural characters. Courtship and guarding behaviour may contain information about male age. It is possible that some experience-dependent performance of displays could be used by females as a measure of age. In black sage grouse, *Centrocercus urophasianus*, females appear to base their choice on features of male behaviour which develop with age (Wiley 1978).

The effect of age on drumming behaviour of stonefly males, *Pteronarcella badia*, was studied by Zeigler and Stewart (1985). Male drumming behaviour is an intersexual calling behaviour for mate location and identification and is produced by tapping the substratum with the abdomen. There was no difference between young and old males for number of beats per signal or beat interval length.

There have been several studies of female choice for male age in different species of insects and some are summarized in Table 2. Woodhead (1986) studied the cockroach *Diploptera punctata* to determine whether there were differences in the courtship behaviour, female response to courtship, and male mating success between old and young males. Older males courted sooner and females responded more quickly to older males. There was no difference in duration of courtship or courtship intensity between young and old males. However, older males were more successful than younger males in competitive mating tests. Male *D. punctata* transfer nutritious material to the female during mating and accumulate this material if they do not have the opportunity to mate which caused older males in this experiment to have more sperm and larger spermatophores than young males. Therefore, in this study, it may not have been male age but the amount of reproductive material that males transferred which affected female choice.

Zuk (1987 b) studied mate attraction of male *G. veletis* and *G. pennsylvanicus* of different ages in an arena experiment where males were suspended over pitfall traps. Females were more likely to approach older than younger males. Similarly, Long *et al.* (1980) conducted mating experiments on fruit flies, *Drosophila melanogaster*, where females were presented with both a young and an old male.

Table 2. Results from some female choice experiments for males age in insects.

Species	Author	Lab (L) or Field (F) Study	Females prefer older males?
Cockroach *Diploptera punctata*	Woodhead (1986)	L	Yes*
Field Cricket *Gryllus veletis*, *G. pennsylvanicus*	Zuk (1987 a)	F	Yes
Fruit Flies *Drosophila melanogaster*	Long *et al.* (1980)	L	Yes**

* not determined if due to amount of reproductive material male transfers.

** male/male competition may have affected results.

Females mated more frequently with older males. However, because of the experimental design, it was not clear if the females mated with older males because they preferred them, or because older males were more successful in male-male competition.

Since some characters can be correlated with others, it is not surprising that age can be correlated with male size, territory size, dominance, and parasite load for example. Of particular interest to this study is the possible correlation between parasite load and age. Perhaps as males age, prolonged exposure to parasites results in higher parasite loads in old males. In adult black grouse, higher infection levels of blood parasites were found in adult relative to young males (Hoglund *et al.* 1992). However, older males may be resistant to parasites and are able to live longer. Borgia and Collis (1989) found that older satin bowerbird males had fewer parasitic lice. Other studies have found no correlation between age and parasite load. Simmons and Zuk (1992) did not find any correlation between male age and gregarine parasite load in *Gryllus bimaculatus.*

CRICKET MATING SYSTEMS

The following section describes the mating systems of various species of crickets. Emphasis is placed on the mating behaviour of *Gryllus integer*, since it is the study animal in this project. Males attract females, exhibit courtship behaviour to secure a mating, and perform post-copulatory mate guarding behaviour.

Mate Attraction

A few species attract mates through chemical signals. *G. ovisopis* has lost the ability to call but can still produce courtship and aggressive chirps. This species has high population densities and communication by antennal contact and olfaction are probably sufficent for mate

attraction. Some Australian crickets (unidentified species) living in caves have lost their acoustic signaling ability and neither males nor females have organs for sound production or reception and seem to use olfaction or contact chemoreception for communication. Other Australian crickets use substrate vibrations for communication rather that acoustic signals (Loher and Dambach 1989).

The most common strategy employed by male crickets to attract female crickets is through song. The song is produced by rubbing the tegmina, the modified upper wings, together. A file on the undersurface of one wing is struck by the scraper on the upper posterior surface of the other wing. The action of rubbing the tegmina together to produce a call is termed stridulation. Stridulation either produces a train of pulses or a pure tone depending on the rate of striking and the properties of the tegmina. The songs of trilling crickets are long trains of similar pulses, while chirping crickets produce songs organized into repeated groups of pulses separated by silent intervals. Context and species-specific information is conveyed by how the pulses are organized into chirps and trills. Males call most frequently at dusk and dawn and it is during these times that females are most receptive to the calls. Both males and females have hearing devices located on the forelegs called tympana that are used to listen to males calls (Alexander and Otte 1967).

Many Grylline crickets occupy burrows and stand in front of the burrow entrance when calling. Male short-tailed crickets, *Anurogryllus muticus*, call near their burrow entrance in a small depression which amplifies and directs the sound up towards flying females. Male mole crickets, *Scapteriscus acletus* and *S. vicinus*, dig burrows with horn-

shaped entrance tunnels which act to amplify the sound of their calling songs (Loher and Dambach 1989).

Some species have evolved alternative strategies to calling. Nighttime calling *G. integer* males are often surrounded by aggregations of females and silent satellite males . These non-calling males intercept approaching females, court them, and try to mate. At dawn, the number of calling males increases as many previously silent crickets begin singing (Cade 1975, 1979, 1980).

Calling *G. integer* males not only attract females, but attract a parasitoid fly, *Ormia ochracea*. The female flies deposit larvae on and around the host. The larvae penetrate the host cuticle and can consume a cricket within seven days. Since satellite males become parasitized less often than singing crickets, the parasitoid files constitute a selective force against calling (Cade, 1975, 1979, 1980). Satellite behaviour has also been seen in *Teleogryllus commodus, T. oceanicus,* and *Gryllodes supplicans* (Sakaluk 1987). Mammals and birds can find and capture singing crickets. Geckos can intercept females that are attracted to calling males. Being silent not only protects a male from predatory attack, but saves noncallers the energy required to sing. However, this is a tradeoff since non-calling males are also not attracting females and therefore suffer reduced mating success (Loher and Dambach 1989). In crickets that communicate acoustically, the first opportunity for females to select potential mates is by using such long range signals. Females prefer longer calling songs in the California field cricket, *G. integer* (also called *G. integer*) (Hedrick 1986).

Courtship Behaviour

Once a female has made a choice, she will approach the chosen male. Sex recognition pheromones are transferred by antennal contact

between the male and the female. *G. bimaculatus* males palpitate the female's body and a pheromone is probably perceived. Sex recognition odours at close range have been demonstrated in *Acheta domesticus, Gryllus integer,* and in other *Gryllus* spp. (Otte and Cade 1976). Conspecific odours are preferred to heterospecific ones.

Once a male establishes that there is a sexually receptive female close to him, he may begin courtship behaviour. Courtship behaviour consists of various displays performed by the male. During courtship behaviour, the male usually performs a courtship song. In field crickets, the courtship song is less species-specific and stereotypic than the highly discriminating calling song (Loher and Dambach 1989).

Most cricket species adopt the evolutionarily primitive female-above-male posture for copulation. All male crickets use externally attached sperm packages (spermatophores) for insemination. In most species of Gryllinae, a spermatophore consists of an ampulla containing sperm, an anchor plate, and a tube. During copulation, the spermatophore is transferred to the female, with the ampulla protruding outside and held in place by the attachment plate slipped into the female above the subgenital plate, while the tube is threaded through the genital chamber into the spermathecal duct aperture. In *Gryllodes supplicans* and in several Australian species of the genus *Teleogryllus,* the spermatophore ampulla is capped with a large fibrous mass which is consumed by the female after mating and may be an important source of nutrition (Alexander and Otte 1967, Sakaluk 1987).

Mating duration is highly variable and can range from only a few seconds for some Nemobiinae, 30 seconds in *Acheta domesticus,* to 90 minutes for *Discoptila fragosoi.* The frequency of mating depends on a variety of factors (Loher and Dambach 1989). Females of some species

stay receptive after mating, whereas females of other species may become defensive. The male's ability for renewed mating depends on the time required to produce the next spermatophore. A female can mate several times and is able to store sperm in her spermatheca (Backus and Cade 1986).

Post-Copulatory Mate Guarding Behaviour

Post-copulatory behaviour is concerned with mechanisms that assure complete transfer of spermatophore contents to ensure egg fertilization (Sakaluk 1987). Males from species with retained spermatophores that are emptied during copulation are concerned with defending the female against copulatory attempts from other males. Some species of crickets have lengthy copulation durations. This may ensure that the sperm will be transferred to the female or it may prevent the female from mating with other males (Loher and Dambach 1989).

More common is the strategy of guarding the female to protect the male's mating efforts. Guarding behaviour is widespread among the Gryllinae and may act to prevent competitors from inseminating the female, to hinder a female from removing the spermatophore, and to monopolize the female for further matings. The idea of guarding a female to stop her from removing the spermatophore before all of the sperm has been transferred to her spermatheca assumes that guarding duration is correlated to the time it takes to deplete the spermatophore of sperm (Simmons 1990).

The male guards a female by maintaining close body contact with the dismounted female, often by touching her with his antennae, and performs various guarding displays. Attempts by the female to leave result in vigorous displays of juddering, turning, and antennal shaking

for example, until physical contact is regained which seems to calm the female (Simmons 1990).

Since the female can exhibit mate choice after mating by removing the spermatophore, the role of guarding may be to protect the female from mating with other males. *G. bimaculatus* females preferentially mate and stay with large males, and several inseminations may occur. However, small males may be abandoned after copulation and the spermatophore is removed before its contents can migrate into the female's spermatheca. The female may make decisions about the quality of the male by assessing the male's guarding ability (Simmons 1986).

The production of a spermatophylax may be a mechanism to protect the males' mating efforts since during its consumption the sperm can migrate into the female's spermatheca before the ampulla is eaten. The metanotal glands of male tree crickets (Oecanthidae) divert the female's attention from the attached spermatophore. If the female does not begin to consume the secretion, she will leave at once and consume the full spermatophore instead (Sakaluk 1984, 1985).

The final stage of reproduction is the laying of eggs by the female. As virgins, females store their eggs in their ovaries. After mating, the female is stimulated to oviposit her eggs and several hundred eggs are deposited into a suitable substrate (Loher and Dambach 1989).

GREGARINE PROTOZOAN PARASITES

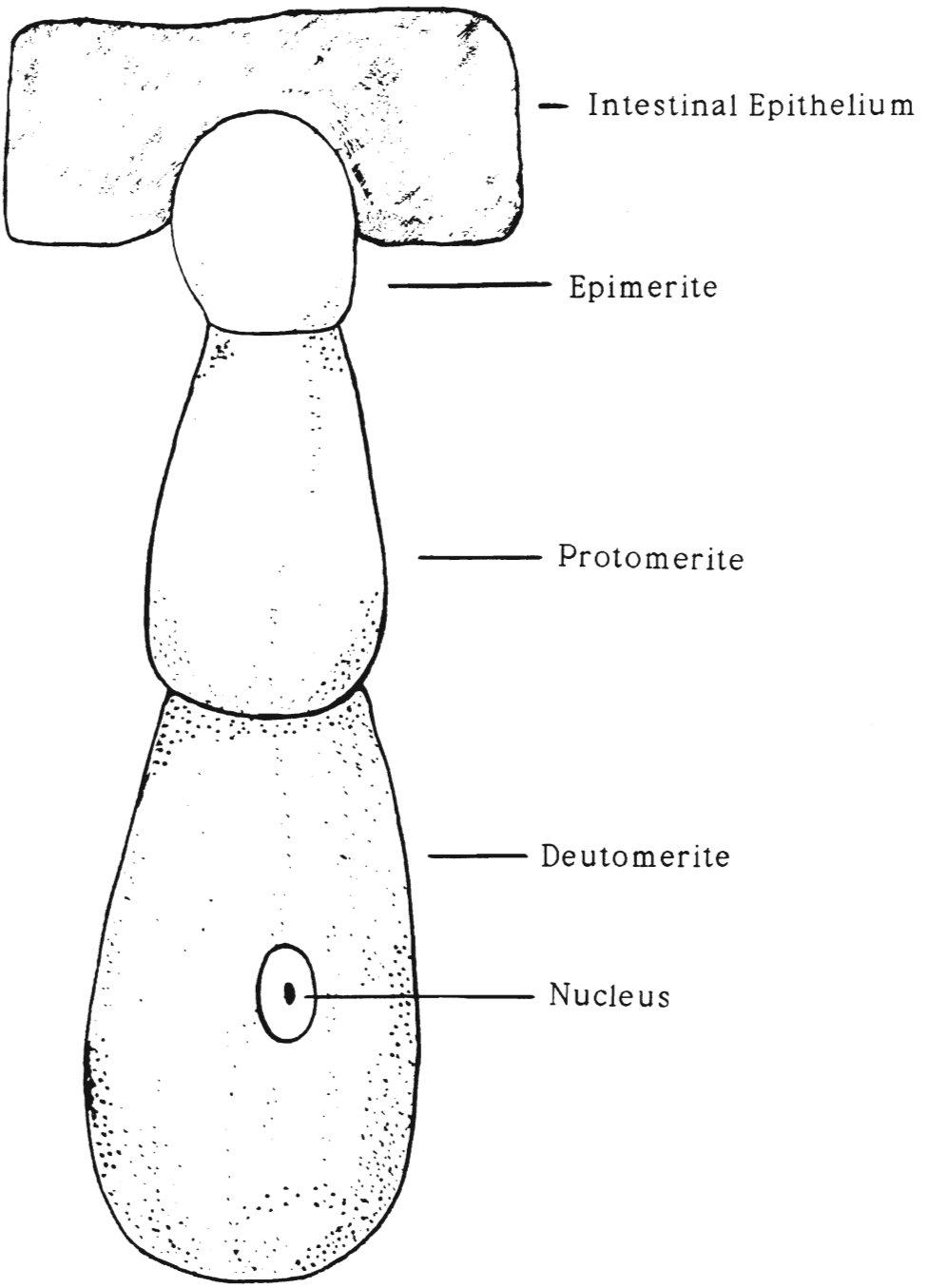
Gregarines (Order Protozoa, Subphylum Sporozoa, Subclass Gregarinia) are intestinal parasites common in many invertebrate species and are usually found in the digestive or excretory systems of arthropods and annelids. Gregarines, from the genera *Gregarina*, are

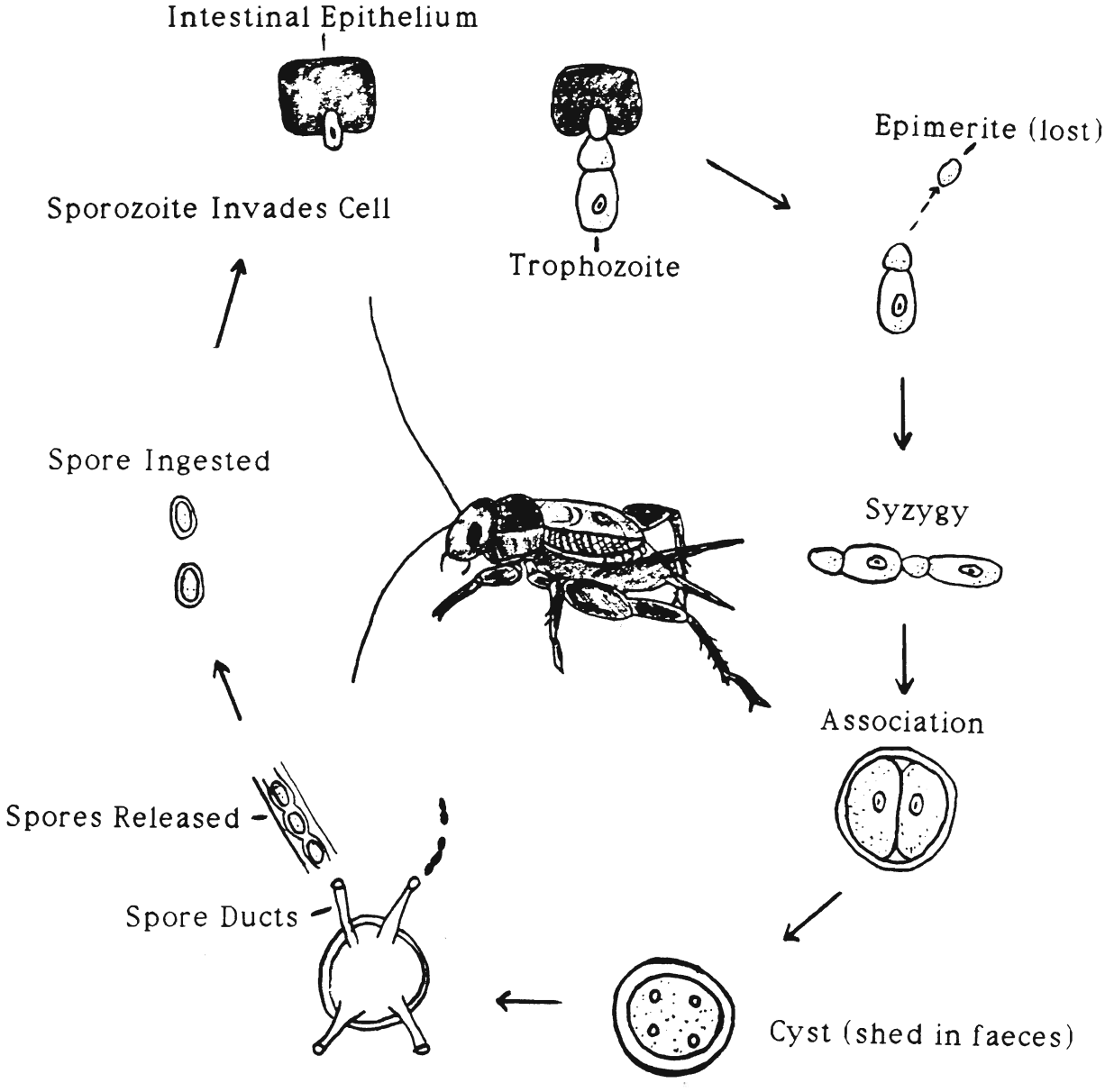
commonly found in the gut of various insects species including beetles, cockroaches, grasshoppers, and crickets (Smyth 1976).

The body of the parasite is divided into three regions the epimerite, protomerite, and deutomerite (Figure 1). The epimerite, or holdfast organelle, is a specialized region which attaches to the host and absorbs nutrients from the host's tissue. The fully developed gregarine is covered with longitudinal ribs which are probably used for locomotion (Vavra and Small 1969).

The life cycle of the gregarine is depicted in Figure 2. Hosts ingest spores which hatch and release sporozoites that penetrate the host epithelial cells of the midgut portion of the digestive tract and develop as trophozoites. The intracellular region grows rapidly. When it reaches 13-16 μm, the protomerite becomes distinct from the deutomerite. Mature trophozoites can reach up to 1 mm in length. When the gregarines become separated from the host cell, they are able to form a sexual association termed syzygy with another, and sometimes more than one, trophozoite (Sprague 1941). At this stage, the trophozoites form a permanent end-to-end association and often move around in this joined condition. The trophozoites then encyst and a thick gelatinous cyst wall is produced. These cysts travel down the digestive tract and are shed in the faeces. In conditions of warmth and humidity, the cysts will sporulate and spores will be released into the environment to be ingested by the next host and the infectious cycle will continue. The life cycle generally takes 11-13 days. Cysts may appear in the faeces 9 days after an initial infection (Smyth 1976).

The synchronization of host and gregarine life cycles has been noted in many species. For example, in *Locusta migratoria*, many cysts are released immediately prior to moulting, when the gut epithelium is


Intestinal Epithelium
Epimerite
Protomerite
Deutomerite
Nucleus


Intestinal Epithelium
Sporozoite Invades Cell
Trophozoite
Epimerite (lost)
Spore Ingested
Syzygy
Association
Spores Released
Spore Ducts
Cyst (shed in faeces)

shed (Smyth 1976). In several species of polychaetes, cyst formation only takes place when the annelid host becomes sexually mature and sheds its sexual products. The synchronization of host and gregarine life cycles is probably under hormonal control (Smyth 1976).

Fitness Effects of Gregarines on their Hosts

Gregarines can have negative affects on their host's condition and viability. In the damselfly *Pyrrhosoma nymphula*, heavy gregarine infestations damage the gut epithelium and expose the host to pathological conditions which weaken infected damselflies. Mark and recapture experiments found that individuals which attained greatest adult age were either lightly infected or uninfected. Gregarine infestation appeared to reduce the longevity of adult damselfies when other conditions were sub-optimal (Abro 1971). Gregarine parasites infect the larva of the beetle *Tenebrio molitor*. When the larvae were grown under optimal conditions of temperature, humidity, and diet, there were no differences between infected and uninfected larvae with respect to length of larval life or final pupal weight. However, when larvae were grown on a sub-optimal diet containing only minimal amounts of vitamins, 33% of the infected larvae failed to pupate, had more difficulty moulting, and weighed less than uninfected larvae (Harry 1967).

Zuk (1987 a, b, c) studied gregarines in field caught *G. pennsylvanicus* and *G. veletis*. The crickets were frozen, thawed, and re-frozen which caused several of the trophozoites to lyse, making quantitative counts impossible. Therefore, trophozoites were qualitatively ranked into categories of light, medium, and heavy parasite loads. Zuk found that gregarines affected the health of the crickets (Zuk 1987 a). Male crickets infected with gregarines had a

reduced reproductive potential because of an inability to rapidly replenish spermatophores (Zuk 1987 b). In *G. pennsylvanicus*, infected males had reduced longevity and increased weight loss relative to uninfected controls (Zuk 1987 a). Also, the developmental period for infected individuals was longer than for uninfected individuals (Zuk 1987 c). Zuk (1988) also showed that *G. pennsylvanicus* and *G. veletis* males with low levels of gregarine infections are more likely to be paired than males with heavy loads.

Simmons (1990) studied the effect of gregarine infection on the guarding behaviour of *G. bimaculatus* and found that infected males had to put more energy into guarding and were also less successful at keeping females close than uninfected males. In this study, parasite load was measured as the total number of cysts found in the gut. Simmons and Zuk (1992) studied the effects of gregarine infections on the calling song structure and pairing success of *G. bimaculatus* and measured parasite load as described above for Zuk (1987 a,b,c, 1988). Parasite load did not affect any of 15 song parameters measured. It was noted that parasitic infection influenced the pairing success of young males, but not old males, as paraitized young males were never found paired with a female.

Simmons (1993) also studied the effect of gregarines on the mating frequency of bushcrickets, *Requena vericalis*. In this study, Simmons artificially infected field caught crickets by feeding them sporulated cysts. Trophozoite levels were determined by rupturing the midgut, releasing the contents into a saline solution, counting the number of trophozoites in a 1 ml subsample, and then multiplying by an appropriate dilution factor. The number of trophozoites found ranged from 0 to 17,500. He found that heavily infected males mated

less frequently than uninfected individuals when fed a low protein diet. However, when fed a rich diet, infected males were able to overcome the constraints imposed by the infection. This study shows that differences in diet quality and variations in parasite loads can significantly affect mating behaviour of crickets.

METHODS

All of the experiments were performed in the laboratory at Brock University, St. Catharines, Ontario, Canada, between January and December 1993. The species used was *Gryllus integer* which was reared in the laboratory from stock cultures obtained in October 1992 from Austin, Texas.

CRICKET REARING

The crickets used in all of the experiments were hatched from eggs laid in laboratory cultures. Laboratory breeding stock was maintained in either plastic containers (35 x 31 x 17 cm) at approximately 28 - 30°C and relative humidity of 50%, or in plastic garbage pails in the laboratory at 27 - 29°C and relative humidity of 30%. The photoperiod was maintained on a 14hr light/10hr dark cycle. Nymphs were given Purina Cat Chow® and water was provided in glass vials plugged with cotton wool. Cardboard egg cartons were provided as shelters. Lab cultures were checked daily and any newly eclosed adults were removed. All adults taken from the cultures were virgins since male crickets do not become sexually mature until they are about five days old (Cade and Wyatt 1984) and most females do not mate until they are more than three days old (Solymar and Cade 1990). Each adult was kept in an individual plexiglas container (16 x 8 x 9 cm) with a cardboard shelter and given food and water *ad libitum*.

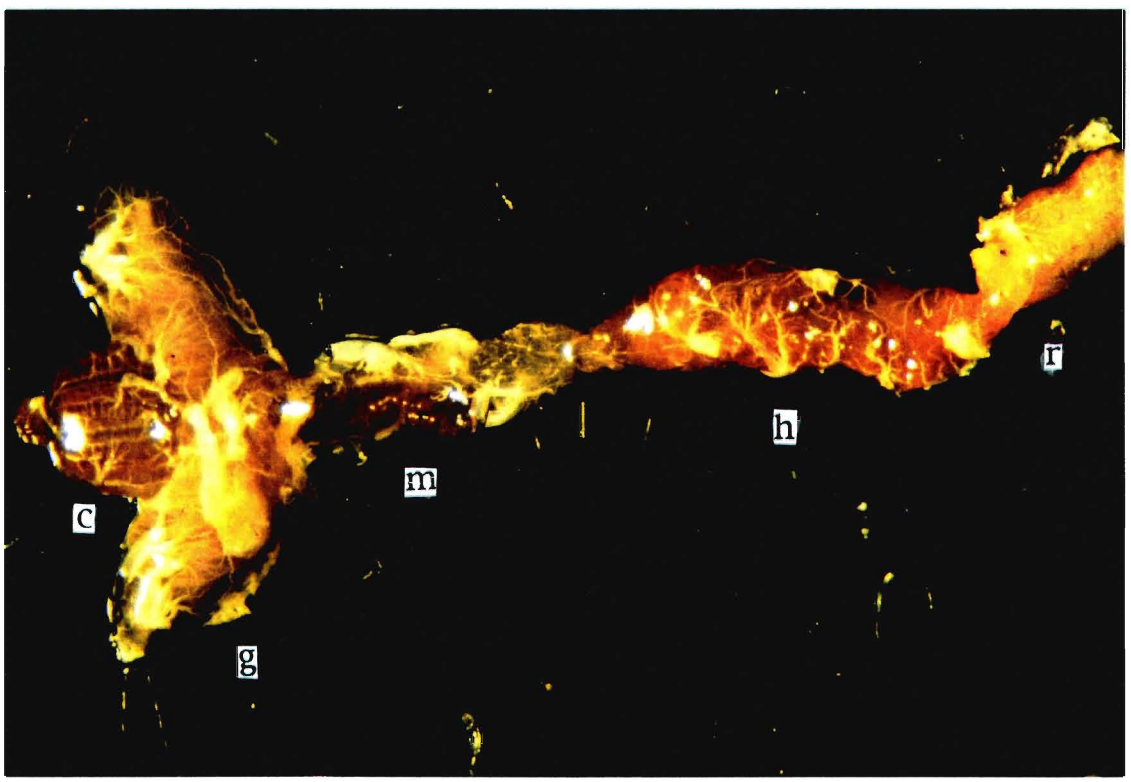
PARASITE LOAD

In order to determine if gregarine parasites influence calling song and mating behaviour in *G. integer*, the parasite levels of individual males were measured. Parasite load was determined by dissecting freshly killed crickets. Individuals were killed by placing them into a jar containing ethyl acetate. This method ensured that trophozoites and

cysts stayed whole in the midgut allowing accurate quantitative counts. Freezing the crickets as a method of killing caused the trophozoites to lyse making quantitative counts impossible (Zuk 1987 a, b, c; personal observations). Preserving the crickets in alcohol also caused the trophozoites to lyse (personal observations).

Dissections were performed in the following way. The wings were removed, the sides of the abdomen were gently cut open along the length of the cricket, and the dorsal cuticle surface was removed. With the aid of a dissecting microscope (x64), a cut was made in the digestive tract above the crop, and the entire digestive tract was removed. The gut tract was placed on a glass slide and examined under the dissecting microscope (x160) (Figure 3). The crop, malphigian tubules, and any fat bodies or pieces of tracheae were gently removed. Then the gastric caecae were pulled open and flattened, one lobe at a time, and examined for gregarine trophozoites and cysts (Figure 4). The entire length of the gut was examined for trophozoites and cysts by carefully breaking apart any material found in the tract. The numbers of trophozoites and any cysts found were noted. Prior to the experiments, males were isolated for 24-48 hrs. and any faeces deposited during this time were collected. The faeces were examined by placing each faecal pellet into a drop of water on a glass slide. Once the pellet had softened, it was gently teased apart and examined for cysts. Records were also kept of weight using a Mettler BB240 electronic balance and morphometric measurements were taken, using Mitutoyo Digimatic calipers, of pronotum length, pronotum width, femur length, and femur width for all individual males (Appendix A, Tables i - iv).

Figure 3. A photograph of the midgut of *Gryllus integer* (x65). Legend: crop (c), gastric caecae (g), midgut (m), hindgut (h), rectum (r).


r
h
m
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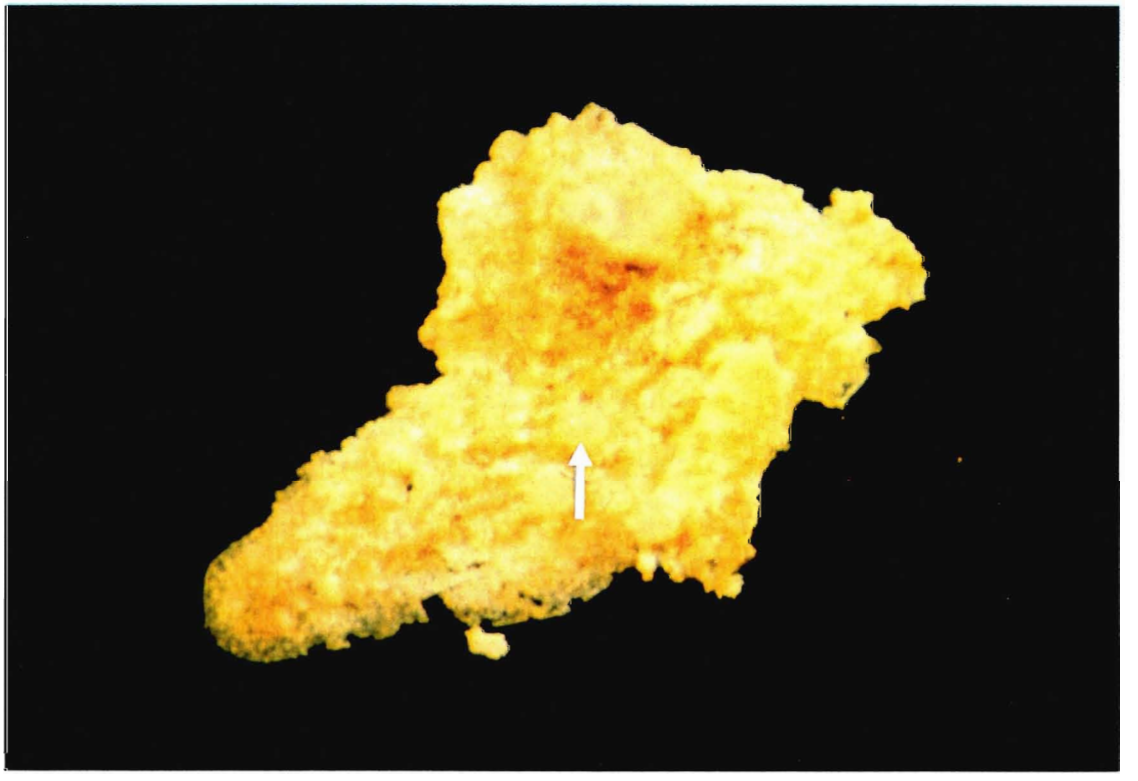
g

Figure 4. a) A photograph of several gregarine trophozoites from the midgut of *Gryllus integer* (x400). b) A photograph of a gregarine cyst in a faecal pellet deposited by *G. integer* (x200).

a)

b)



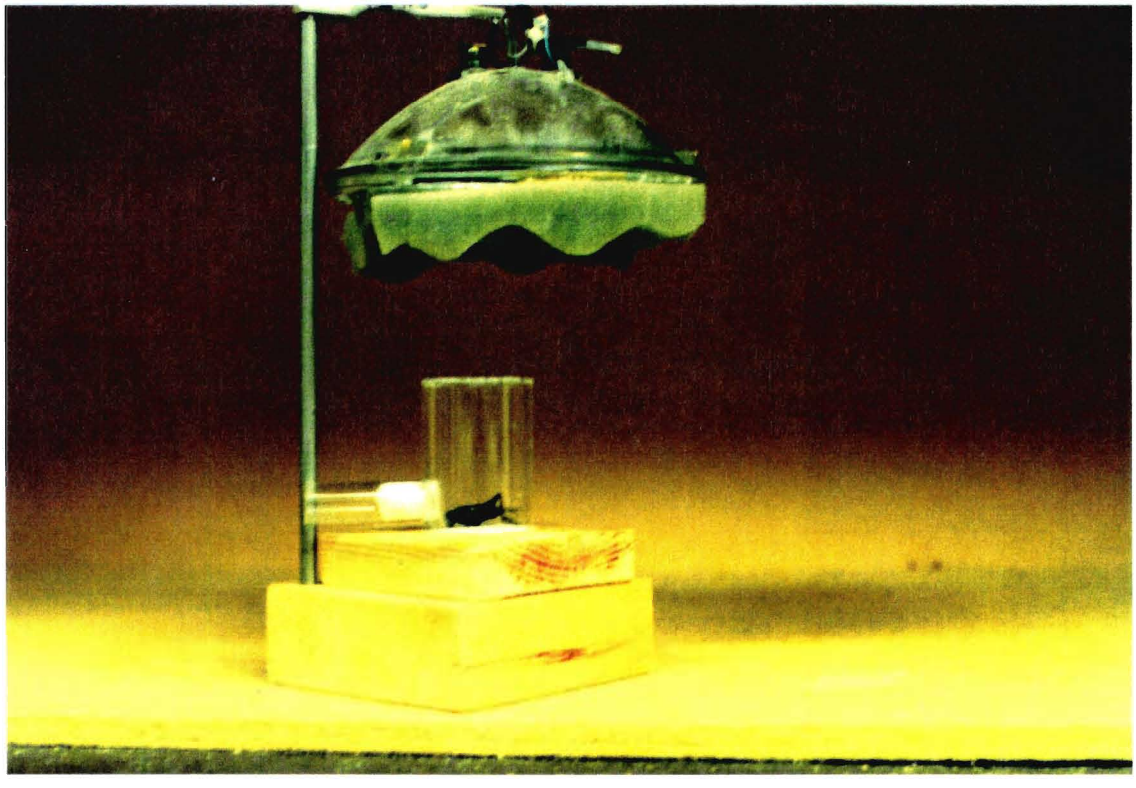
CALLING SONG

To study the effects of gregarine parasite infections on calling song, the calling songs of male crickets were recorded in the laboratory. The songs of individual male crickets were analyzed by a digital signal processing network (DSPN) designed by the Electronics Shop at Brock University. The DSPN is loaded with application programmes through an IBM PC computer. It collects sound impulses produced by crickets and digitizes the recordings for processing by the computer. The DSPN is equipped with a five channel microphone amplifier module which receives signals from five microphone elements (Archer electric #270 090 PC) which are placed inside small parabolic shells. Male crickets are placed inside individual tubular plastic chambers (3.5 x 5 cm) with wire mesh tops and these are then placed beneath the parabolic shells (Figure 5). To prevent males from hearing the calls of other males and to reduce recording interference from other sounds, the recording chambers were separated by particle board.

The DSPN monitored a maximum of five crickets each night for singing activity by means of a polling mechanism. Each recording chamber was monitored in sequence for calling activity for a period of 10 seconds. If no calling was recorded during this time, the DSPN automatically continued polling to the next station. If calling activity was recorded at the station the polling time was extended to 60 seconds before resuming regular polling. All song data collected were stored on the hard drive of the PC.

The DSPN required two thresholds to be entered in order to analyze song. An upper threshold defined the start of the active pulse width and the pulse period, and the lower threshold defined the end of the active pulse width. The end of the pulse period was determined by

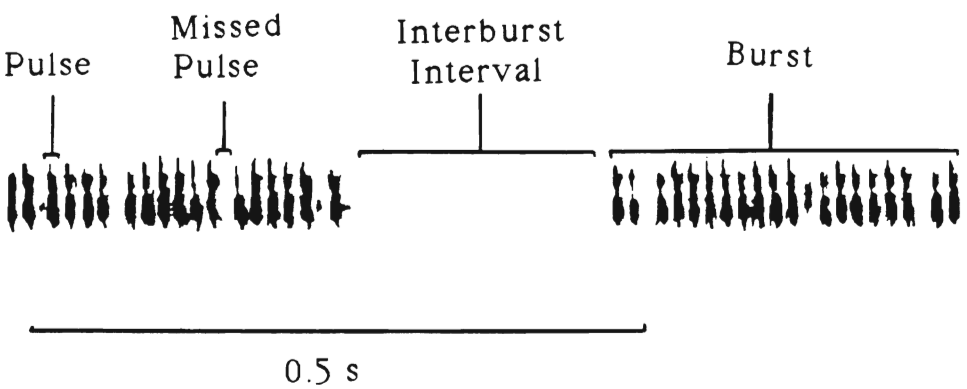
Figure 5. A photograph of the apparatus used to contain males during song recordings. The parabolic shell is lowered over the plastic chamber during recordings.



the next encounter of the upper threshold. These parameters were used to calculate 6 song structure characteristics (Figure 6). Pulse rate was defined as the reciprocal of pulse period and was converted to number of pulses per second. When a cricket drops a pulse, the pulse period approximately doubles. As the sample size increases, a second peak appears on the pulse rate frequency distribution, its height being representative of the number of dropped pulses. The proportion of dropped pulses was expressed as a percentage (frequency of dropped pulses divided by the modal frequency of the distribution, multiplied by 100). Burst duration was defined as the duration of a train of pulse periods, separated from other trains by more than 102.4 ms. Interburst interval was defined as a duration between two pulse periods greater than 102.4 ms but less than 1638.4 ms. Pulse width was calculated as the time between consecutive upper and lower thresholds. Number of pulses per burst was defined as the number of active pulse widths within a burst duration (expressed as an integer between 1 and 127). The majority of the songs which were recorded contained greater than 10,000 pulses of song. This amount of song recording has been shown to generate an adequate sample of song for analysis (Souroukis, 1990).

Males were isolated in individual recording chambers 48 hrs. before the experiment to allow them to acclimate to the chamber. The chambers were lined with filter paper to collect any faeces deposited during this time and throughout the song recording experiment. Crickets were placed into the DSPN between 9:00 am and 10:00 am and monitored for calling song for 24 hours. A maximum/minimum thermometer was used to record the temperature in the laboratory over each recording session since song characteristics are known to be affected by changes in temperature (Walker 1962). After their calling

Figure 6. A representative spectrogram of *Gryllus integer* calling song indicating the song parameters studied.


Pulse
Missed Pulse
Interburst Interval
Burst
0.5 s

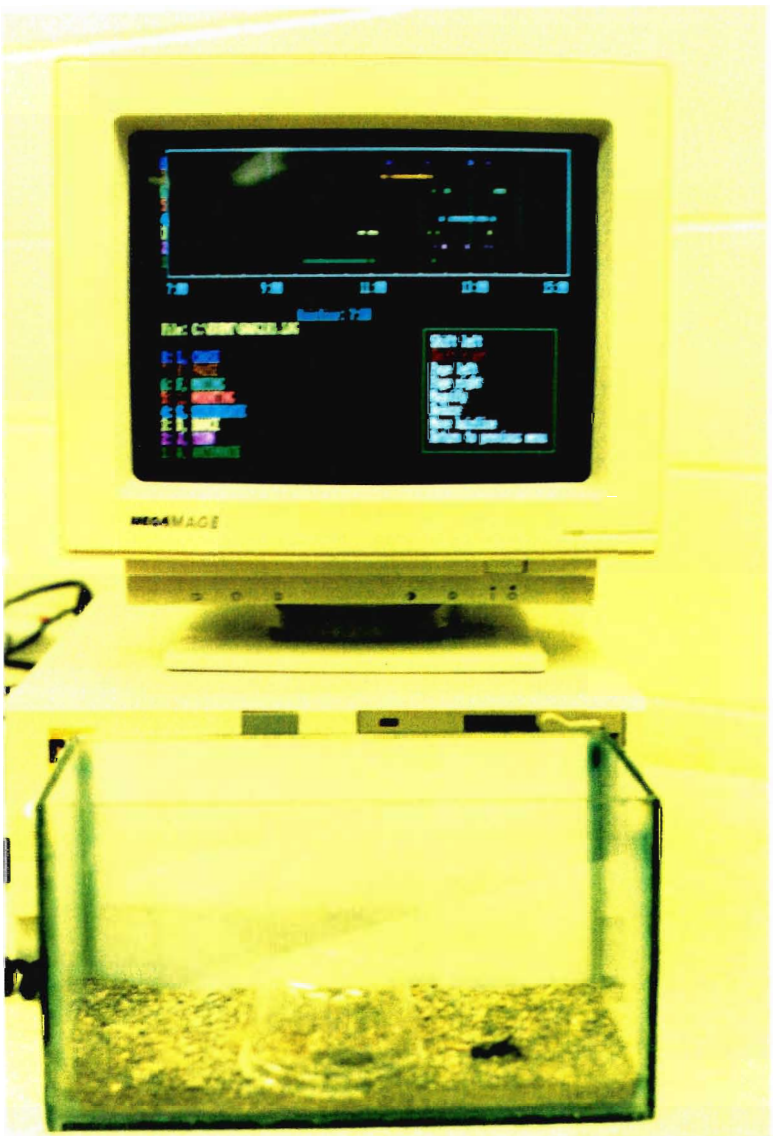
songs were recorded, males were dissected 1 to 4 hours after removal from the DSPN and examined for gregarine parasite infections. All crickets were weighed, dissected, and measured as described above.

COURTSHIP AND POST-COPULATORY GUARDING BEHAVIOUR

Courtship and guarding behaviour were observed to determine if various parameters of these behaviours are affected by parasite infection levels. Males used in the observations were isolated 24 hours prior to trials in a container lined with filter paper. Faeces deposited on the paper were collected and examined for gregarine cysts. For observations on courtship and guarding, a single male was placed inside a glass terrarium (28 x 15 x 15 cm) lined with sand and was then covered with a plastic cup (Figure 7). A virgin female 15-25 days old and of approximately equal size, was also placed inside the terrarium. After five minutes, the plastic cup covering the male was removed and observations of courtship behaviour began. All observations were recorded using the programme Eventlog® on an IBM PC computer. This programme allowed the keyboard to become an event recorder and specific movements performed by the males were assigned individual keys. As the male performed each behaviour, the assigned key was depressed. The programme monitored the total time that each key was depressed and this corresponded to the total time duration that the male spent performing the relevant behaviour.

Male courtship behaviour consists of a series of displays which may result in a successful mating. Courtship behaviour has been described to some extent by Alexander and Otte (1967) for several spieces of field crickets and by Boake (1984) for the gregarious cricket *Amphiacusta maya*. Courtship behaviour is initiated when antennal contact is made between a male and a female. If the female remains

Figure 7. The terrarium where behavioural observations were conducted and an example of the output from the Eventlog programme.


MEGAIMAGE

immobile after antennal contact, the male turns his body away, and begins to judder vigorously by rocking on his legs forward and backward. At this time the male may place his antennae in a "V" position in front of his body and shake them vigorously in the air. The male may raise his wings and produce courtship song or keep them raised without stridulating. The courtship song is almost always necessary to stimulate the female to mount the male (Alexander and Otte 1967). To allow the female to mount, the male lowers his body and moves his wings to the sides of his body. If the female remains mounted, the male will then transfer his spermatophore between the base of her ovipositor and subgenital plate. Not every mounting results in a successful mating. If a mounting is not successful, the male usually resumes courtship behaviour. If the female moves away from the male, he searches to find her to make antennal contact again and then resumes courtship displays. Behavioural terms used in this study were based on personal observations and from previous information published on similar species of crickets (Alexander and Otte 1967, Boake 1984, Simmons 1987). The 9 displays recorded for male courtship are described in Table 3. Each cricket pair was observed for 20 min, or until a successful mating occurred.

Males initiate post-copulatory guarding behaviour after a successful mating. The male may perform several displays which are similar to the courtship displays described above. He may judder, antennate the female, shake his antennae, raise wings without stridulation, search for the female, turn, and groom his antennae by passing them through his mandibles (Simmons 1990). The 8 displays recorded for male mate guarding are described in Table 4. All males that mated successfully were observed for 10 minutes for mate

Table 3. The courtship behaviour displays of the male field cricket, *Gryllus integer*.

Display	Description
antennate	male touches female with antennae
judder	male rocks forward and backward on legs
turn	male turns towards or away from female
pause	male remains motionless
search	male searches for female
raise wings	male raises tegmina without stridulation
stridulate	male performs courtship song
shake antennae	male vigorously shakes antennae
spermatophore transfer	male spermatophore is transferred to female

Table 4. The post-copulatory guarding displays of the male field cricket, *Gryllus integer*.

Display	Description
guard antennate	male touches female with antennae
guard judder	male rocks forward and backward
guard turn	male turns towards or away from female
guard pause	male remains motionless
guard search	male searches for female
guard raise wings	male raises tegmina without stridulation
guard shake antennae	male vigorously shakes antennae
guard groom	male passes antennae through mandibles

guarding behaviour. Although guarding in field crickets can take 40-60 min, it is during the first 10 min that the male achieves the maximum rate of increase in fertilization (Simmons 1987). The rate of increase in fertilization success, when sperm are in competition, follows the law of diminishing returns with a decline being reached after 10 min of spermatophore attachment. Attachment times of less than 10 min result in the transfer of sperm loads that suffer considerable long term dilution consequences. Therefore, it is most important for the male to guard the female closely immediately after mating when sperm transfer is greatest.

DIETS

The interaction between parasite load and diet was examined by keeping all males on one of two diets, *ad libitum* (AL) or nutritionally deprived (ND) for the calling song and courtship and guarding behaviour experiments. Crickets on the AL diet were provided with Purina Cat Chow® and water *ad libitum* for their entire lives. Males on the AL diet were various ages in all of the experiments. Another group of males were maintained on the ND diet to investigate if crickets deprived of nourishment were more affected by gregarine infections. These crickets were given Purina Cat Chow® and water *ad libitum* as nymphs and for the first 10 days of adulthood, and were then deprived of all food and given only water for the next five days before being used in an experiment. Due to the experimental design all crickets reared on the this diet were 15 days old when their songs were recorded and courtship and guarding behaviours observed.

The calling songs of 88 males on the AL diet were recorded using the DSPN and of these, 46 males recorded at least 10,000 pulses of song. Their ages ranged from 15 to 33 days. The songs were recorded at a

mean temperature of 23°C ± 1°C with a maximum temperature of 24°C and minimum temperature of 21°C. The calling songs of 17 males on the ND diet were recorded and of these, 12 males recorded at least 10,000 pulses. The songs were recorded at a mean temperature of 24° ± 0.4°C with a maximum temperature of 24°C and minimum temperature of 23°C.

The mating behaviour of 63 males on the AL diet were observed and recorded. These males ranged in age from 14 to 33 days. Of these males, 51 were observed for courtship and guarding behaviour. The mating behaviour of 68 males on the ND diet were observed and of these, 44 males performed courtship and guarding behaviour. All males in the ND group were 15 days old.

ANALYSIS

All statistical analyses were performed on Apple Macintosh SE or IIx using Statsview 512+ software and Systat 5.1 software (Wilkinson 1989). All data distributions were tested for normality using the Kolmogorov-Smirnov goodness of fit test and most proved to be non-normal. A variety of transformations were applied to normalize the distributions, but none was successful at transforming most of them, including parasite load distributions. Therefore non-parametric statistics were used for data analysis.

To determine the best way to measure parasite load, Spearman Rank correlations were performed on total number of trophozoites in the gut vs. total number of cysts in the faeces in AL and ND diet males in all experimental groups.

Since song parameters can be affected by changes in temperature, all song data were tested to determine if they varied with temperature. Song parameters were first tested for normality using the Kolmogorov-

Smirnov goodness of fit test. Transformations were applied to those parameters that showed non-normal distributions (AL diet: pulse rate, burst duration, pulses per burst, and percent missing pulses and ND diet: pulses per burst). Regression analysis on song data vs. temperature revealed those parameters that were affected by temperature (AL diet: pulses rate and interburst interval). For these parameters, the residual values were used in later analysis.

Two-way ANOVAs on ranked data (Conover and Iman 1981, Zar 1984) were performed to determine if any of the six song parameters measured was affected by total parasite load, age, or the interaction between parasite load and age in the AL diet group. Since all males on the ND diet were the same age, Spearman rank correlations were conducted to compare the effect of parasite load on the various song characteristics for the ND diet males.

Mann-Whitney U tests were performed to determine if parasite load, or age in the AL diet group, differed between males that mated vs. males that did not mate or if either affected mating success, latency to courtship, or total duration of courtship.

Two-way ANOVAs on ranked data were performed to determine if the total time, or proportion of time, that a male performed each courtship or guarding display was affected by total parasite load, age, or the interaction between parasite load and age in the AL diet group. Spearman rank correlations were performed to evaluate the effect of parasite load on the total duration and percent duration of each courtship display and total duration of each guarding display.

The conservative sequential Bonferroni test of significance was also applied to data where significant differences were found. These additional results are given in Appendix B, Tables v. - viii.

RESULTS

ESTIMATION OF PARASITE LOAD

Parasite load was estimated by separately counting all trophozoites found in the gut and cysts in the faeces. The relationships between trophozoites in the gut and cysts in the faeces for all males who were parasitized in the calling and mating experimental populations in each of the two diet groups are given in Figure 8. In the AL diet group including both calling and mating behaviour males, the relationship between trophozoites and cysts was significant (r_s=0.5, p=0.0001, n=101). However this relationship was not significant in the ND diet males (r_s=0.2, p=0.93, n=54). These results suggest that measuring parasite load by only counting cysts in the faeces is not accurate. Therefore, in this study total parasite load was estimated by summing total trophozoites and cysts found in the gut with total cysts in the faeces for each male. This measure of total parasite load was used in all further anaylses.

PARASITE FREQUENCY

The frequency distributions, means, and ranges of parasite loads were calculated for all groups of males in the experiments. The frequency distribution of total parasite load for all AL diet males whose calling songs were recorded is given in Figure 9 (a). Parasite load in this group ranged from 0 to 2474 parasites per individual. Mean parasite load was 87 ± 365 (n=88) with many individuals having no gregarines (48%). The parasite load in males who gave more that 10,000 pulses of song ranged from 0 to 2474. Mean parasite load was 144 ± 490 (n=46) and many individuals had no parasites (48%). The frequency distributions of total parasite load from the mating behaviour experiment for all AL diet males and mated males are also given in

Figure 8. The relationship between trophozoites in the gut and cysts in the faeces for all calling and mating behaviour males on the a) Ad Libitum diet and b) Nutritionally Deprived diet.

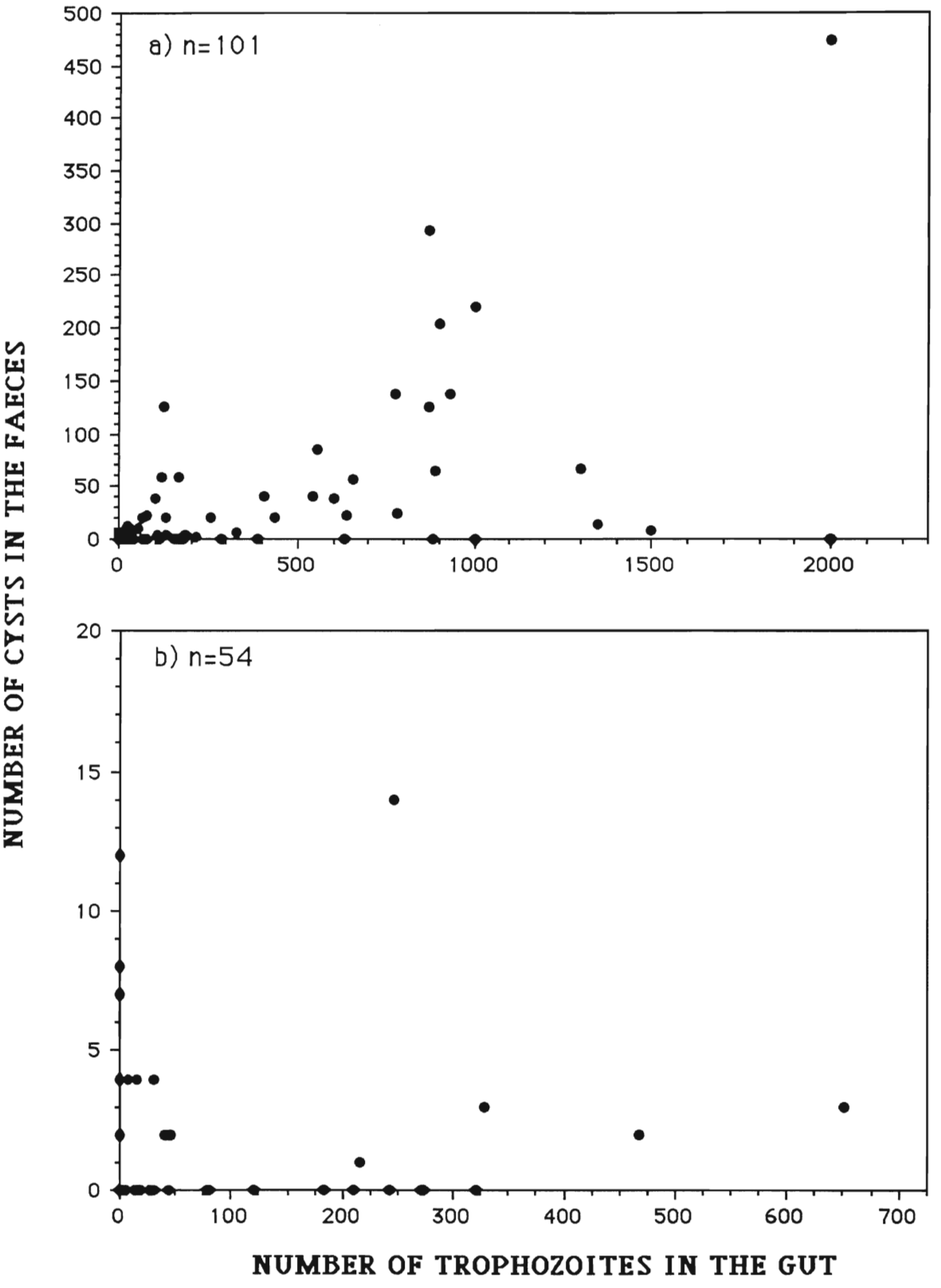

a) n=101
b) n=54
NUMBER OF CYSTS IN THE FAECES
NUMBER OF TROPHOZOITES IN THE GUT
0
50
100
150
200
250
300
350
400
450
500
1000
1500
2000
5
10
15
20
200
300
400
600
700

Figure 9. Frequency distribution of parasites in the Ad Libitum diet group for a) all calling males, b) mating behaviour males, and c) mated males.

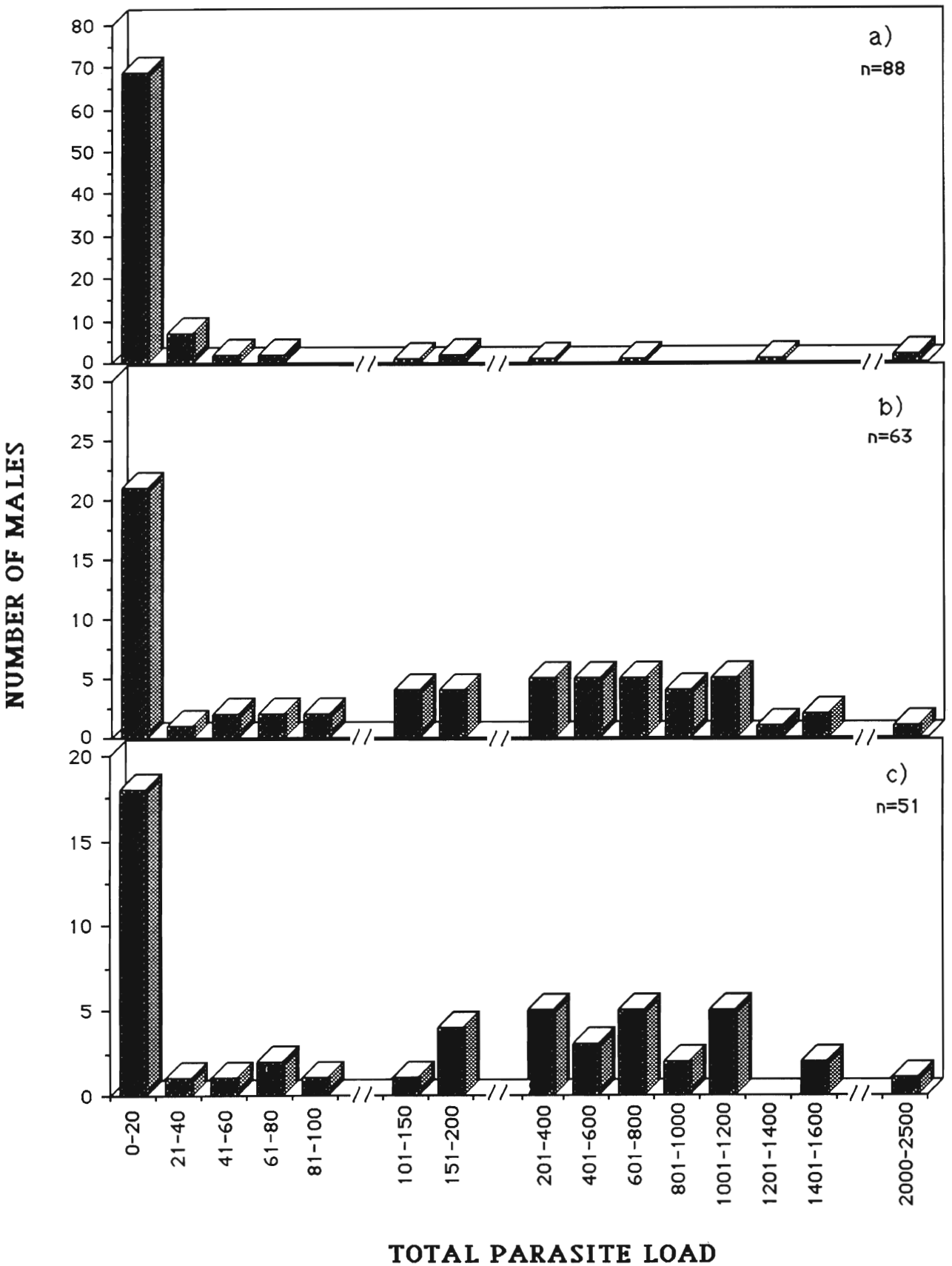

a)
n=88
b)
n=63
c)
n=51
NUMBER OF MALES
TOTAL PARASITE LOAD
0-20
21-40
41-60
61-80
81-100
101-150
151-200
201-400
401-600
601-800
801-1000
1001-1200
1201-1400
1401-1600
2000-2500

Figure 9 (b and c). The mean parasite infection level for all AL diet males observed in the mating experiment was 378 ± 483 (n=63) with a range from 0 to 2003. The mean parasite infection level for AL diet males that mated was 390 ± 496 (n=51) with a range from 0 to 2003.

The frequency distribution of total parasite load for singing males on the ND diet is given in Figure 10 (a). Parasite load ranged from 0 to 470. The mean level was 68 ± 136 (n=12) and 17% of the males had no parasites. The mean parasite infection level for all ND males observed in the mating experiment was 63 ± 127 (n=66) with a range from 0 to 656 parasites. The frequency distribution of total parasite load for this group is given in Figure 10 (b). The mean parasite infection level for ND males that mated was 45 ± 104 (n=44) with a range from 0 to 470 parasites and the frequency distribution is given in Figure 10 (c).

AGE AND PARASITE LOAD

Males in the AL diet group were various ages. Therefore, the relationship between male age and total parasite load was investigated for both AL diet groups. No relationship was found between male age and total parasite load for AL diet males whose songs were recorded (r_s=-0.05, p=0.77, n=88) or for males who sang for at least 10,000 pulses (r_s=-0.05, p=0.76, n=46). These relationships are illustrated in Figure 11. However, a significant correlation was found between male age and total parasite load for males in the mating behaviour experiment. Figure 12 illustrates this relationship for these males. A significant correlation between male age and total parasite load was found in all AL diet males observed (r_s=0.4, p=0.005, n=63), unmated males (r_s=1, p=0.03, n=12), and mated males (r_s=0.3, p=0.02, n=51) with older males having higher parasite loads.

Figure 10. Frequency distribution of parasites in the Nutritionally Deprived diet group for a) all calling males, b) mating behaviour males, and c) mated males.

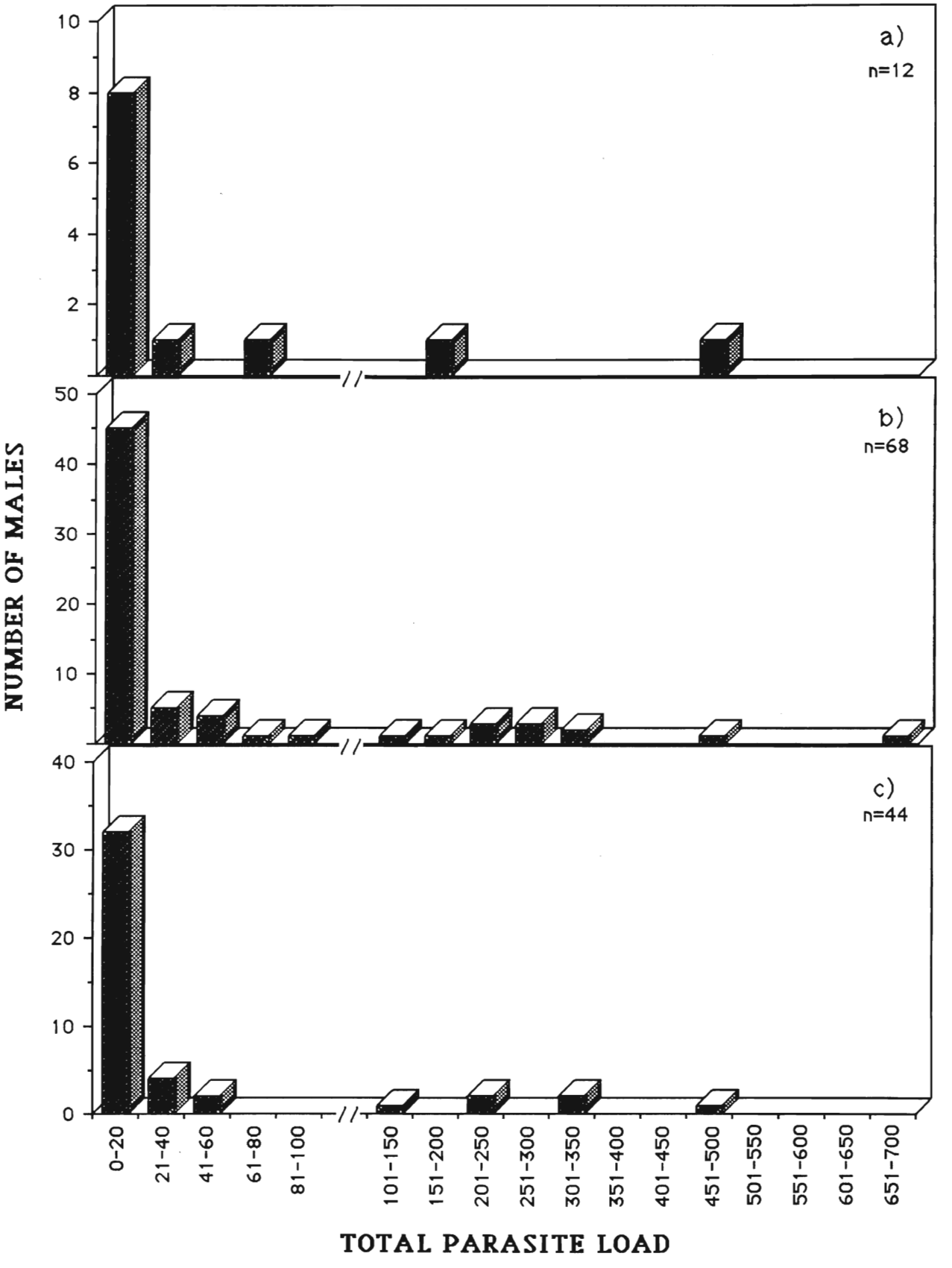

a)
n=12
b)
n=68
c)
n=44
NUMBER OF MALES
TOTAL PARASITE LOAD
0-20
21-40
41-60
61-80
81-100
101-150
151-200
201-250
251-300
301-350
351-400
401-450
451-500
501-550
551-600
601-650
651-700

Figure 11. The relationship between male age and total parasite load in the Ad Libitum diet group for a) all calling males and b) males that produced at least 10,000 pulses.

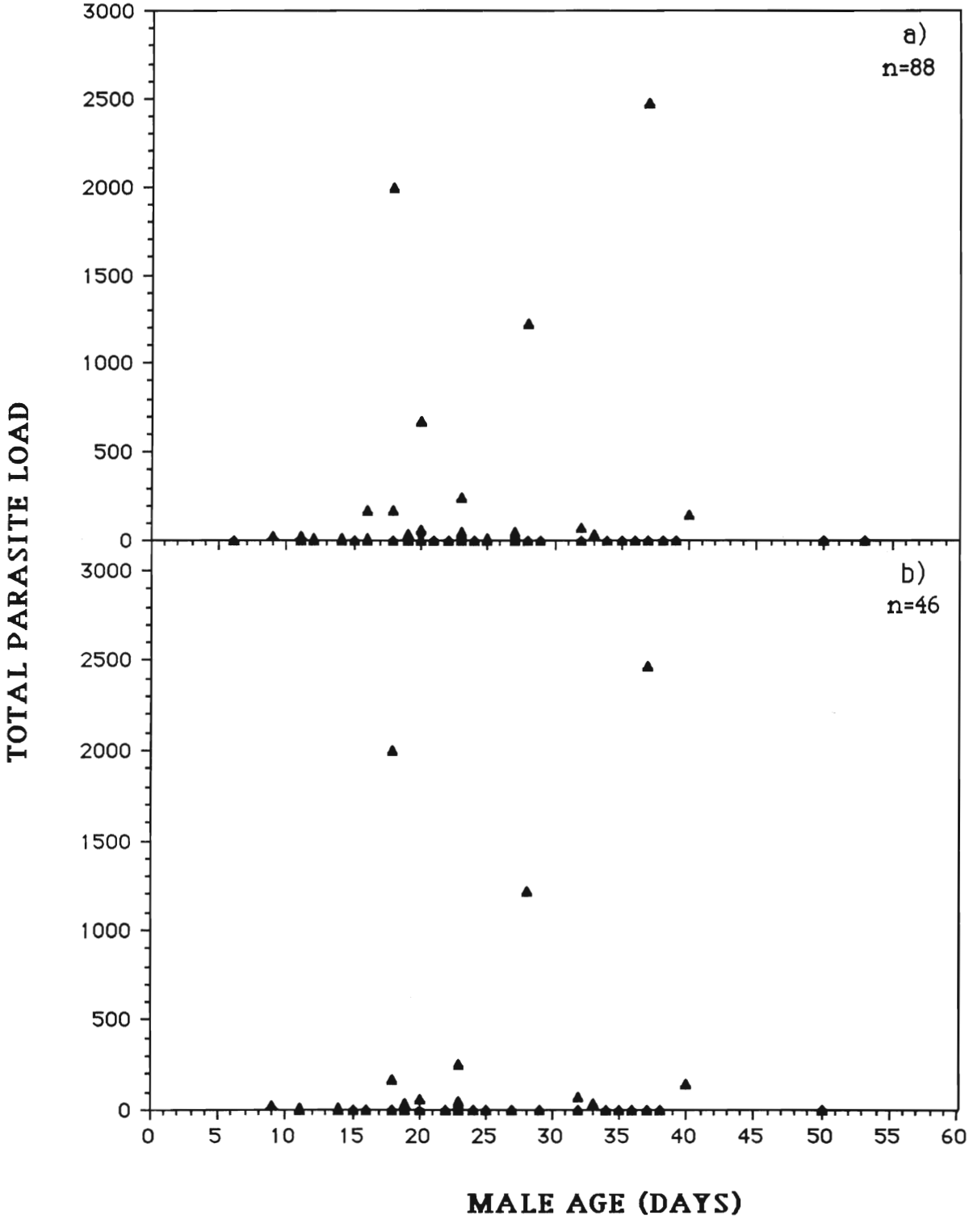
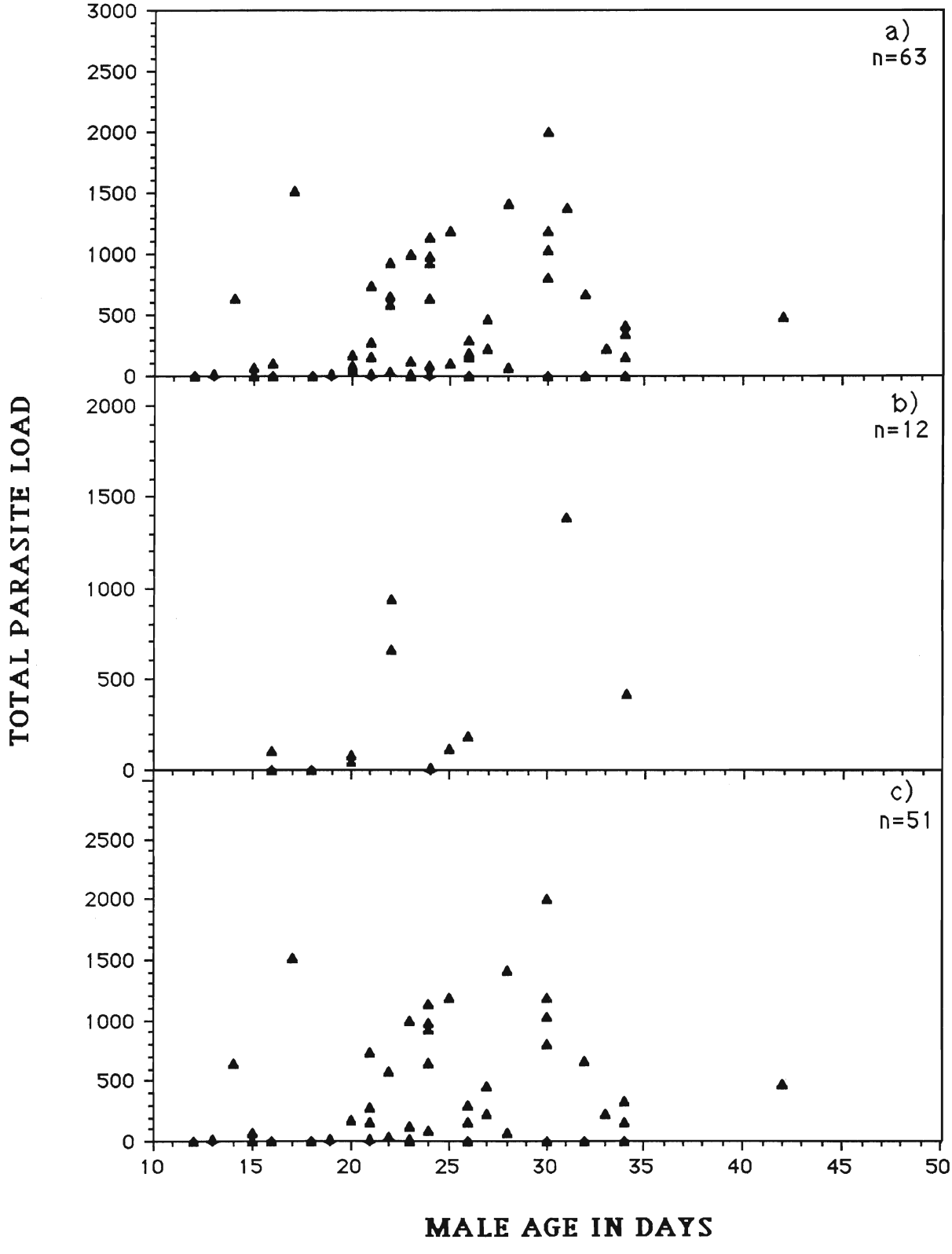

a)
n=88
b)
n=46
TOTAL PARASITE LOAD
MALE AGE (DAYS)
0
500
1000
1500
2000
2500
3000
0
5
10
15
20
25
30
35
40
45
50
55
60

Figure 12. The relationship between male age and total parasite load in the Ad Libitum diet group for a) all mating behaviour males, b) unmated males, and c) mated males.


a)
n=63
b)
n=12
c)
n=51
TOTAL PARASITE LOAD
MALE AGE IN DAYS
0
500
1000
1500
2000
2500
3000
10
15
20
25
30
35
40
45
50

CALLING SONG PARAMETERS

Ad Libitum Diet

Of the 88 males whose calling songs were recorded, 46 recorded at least 10,000 pulses of song and were used in the song analysis. Song parameters were analyzed with respect to the intensity of parasite infection and male age. For this and subsequent analyses, parasite load was divided into 3 groups: low (0 to 100 gregarines, n=23), medium (101 to 500 gregarines, n=12), and high (501 to 2500, n=16). Age was also divided into 3 groups: young (12 to 20 days, n=13), medium (21 to 25 days, n=18), and old (26 to 42 days, n=20). The mean age of calling males was 24 ± 9 days (n=46) with a range from 9 to 50 days. Table 5 shows the mean duration of the 6 song parameters and the F and p values from a 2-way ranked ANOVA. None of the song parameters was significantly correlated with total parasite load or age.

Nutritionally Deprived Diet

The calling songs of 17 ND males were recorded, and of these 12 gave at least 10,000 pulses of song. These males were used in the rest of the analysis. Song parameters were analyzed for effects of parasite infections. Table 6 summarizes the mean duration of the 6 song parameters and the r_s and p values. None of the song parameters was significantly correlated with total parasite load for the ND males.

COURTSHIP BEHAVIOUR

Mating Success

Ad Libitum Diet

The effect of parasite load on whether a male mated or not was analyzed in the AL diet group. Of the 63 males tested, 51 (81%) successfully mated. Males that mated were compared to males that did not mate with respect to total parasite load. The mean parasite

Table 5. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the six song characteristics in Ad Libitum diet males. Parasite load and age were divided into 3 categories (low, medium, high), N=46.

Song Characteristics	$\bar{x}$ ± s.d. (s)	Parasite		Age		Interaction	
		F	p	F	p	F	p
Pulse Rate (# pulses/sec.)	62 ± 7	0.00	1.0	2.29	0.11	1.22	0.32
Pulse Width (ms)	8 ± 3	0.14	0.86	0.76	0.47	0.75	0.56
Burst Duration (ms)	448 ± 150	0.03	0.97	0.57	0.57	0.14	0.97
Pulses/Burst	27 ± 11	0.07	0.93	0.88	0.42	0.09	0.99
Interburst Interval (ms)	270 ± 66	0.37	0.70	0.77	0.47	0.94	0.45
% Missing Pulses	17 ± 11	0.10	0.90	0.36	0.70	0.23	0.92

Table 6. Results from Spearman Rank correlations of parasite load and six song characteristics in Nutritionally Deprived diet males, N=12.

Song Characteristics	$\bar{x}$ ± s.d. (s)	r_s	p
Pulse Rate (#pulses/s.)	66 ± 4	-0.95	0.34
Pulse Width (ms)	8 ± 2	-0.19	0.85
Burst Durations (ms)	450 ± 206	1.58	0.11
Pulses/ Burst	29 ± 15	1.20	0.23
Interburst Interval (ms)	312 ± 53	1.36	0.17
% Missing Pulses	13 ± 5	0.19	0.85

infection level for AL diet males that mated was 390 ± 496 (n=51) with a range from 0 to 2003. For those males that did not mate, the mean infection level was 328 ± 444 (n=12) with a range of 2 to 1384. There was no significant difference between males that mated and males that did not mate with respect to parasite load (z=-1, p=0.49).

Since males in this group were of various ages, this analysis was repeated to study the effects of age. The mean age of all AL diet males was 24 ± 6 days with a range in age from 12 to 42 days. The mean age of the mated males was 24 ± 6 days and the mean age for unmated males was 23 ± 6 days. There were no differences in age between males that mated and males that did not mate (z=-0.1, p=0.88). In this group, neither parasite load nor age affected whether a male mated or not.

Nutritionally Deprived Diet

Males that mated were compared to males that did not mate with respect to total parasite load in the ND diet group. Of the 66 males tested, 44 (67%) mated. The mean parasite infection level for ND diet males that mated was 45 ± 104 (n=44) with a range from 0 to 470 parasites. For males that did not mate, the mean parasite load was 98 ± 160 (n=22) and ranged from 0 to 656 parasites. There was no significant difference between these two groups of males with respect to parasite load (z=-1, p=0.22). Total parasite load did not affect whether a male mated or not.

Time To Mating

The effect of parasite load on total time that a mated male was with a female (from the time the male was introduced to the female until the end of the mating) was analyzed for both diet groups.

Ad Libitum Diet

The mean total time that an AL diet male was with a female was 389 ± 258 s (n=51) with a range of 77 to 1250 s. The total time that a male was with a female was not affected by parasite load (F=1.10, d.f.=2,42, p=0.34), or age (F=1.30, d.f.=2,42, p=0.28), and there was no interaction between parasite load and age on the total time a male was with a female (F=1.17, d.f.=4,42, p=0.34).

Nutritionally Deprived Diet

The effect of parasite load on the total time that a male was with a female was analyzed for the ND males. The mean total time for the ND males was 259 ± 215 s (n=44) with a range of 48 to 904 s. The total time that a male was with a female was not affected by parasite load (r_s=-0.2, p=0.28) for the ND males.

Latency to Initiate Courtship

The time that a male took to initiate courtship was analyzed with respect to parasite load for males in both diet groups.

Ad Libitum Diet

The mean time that an AL diet male took to initiate courtship was 174 ± 201 s with a range of 2 to 1203 s. The time that a male took to initiate courtship was not affected by parasite load (F=0.31, d.f.=2,42, p=0.74), age (F=0.99, d.f.=2,42, p=0.38) or by the interaction between parasite load and age (F=0.58, d.f.=4,42, p=0.68) in the AL group.

Nutritionally Deprived Diet

In the ND diet group, the mean time a male took to initiate courtship was 131 ± 155 s with a range of 1 to 664 s. The time that a male took to initiate courtship was not affected by parasite load (r_s= -0.05, p=0.77) in the ND males.

Courtship Duration

The total time that a male took to court a female successfully, from the start of courtship until mating, was analyzed for both diet groups.

Ad Libitum Diet

Males in the AL diet group took a mean time of 217 ± 195 s with a range of 40 to 912 s to court a female. Courtship duration was not affected by parasite load ($F=1.03$, d.f.=2,42, $p=0.37$) or by age ($F=0.86$, d.f.=2,42, $p=0.43$). However, the interaction term between parasite load and age with respect to courtship duration was significant ($F=3.42$, d.f.=4,42, $p=0.02$). Multiple post-hoc comparison tests failed however to detect any significant differences between any pair of means.

Nutritionally Deprived Diet

The mean courtship time for the ND males was 128 ± 130 s with a range of 26 to 675 s. The analysis showed no significant effects of parasite load on courtship duration ($r_s=-0.01$, $p=0.94$).

Total Duration of Courtship Displays

Male courtship behaviour can be divided into 9 distinct courtship displays. The total time spent performing each display was variable among males (Appendix C, Figures i, ii) and was analyzed with respect to total parasite load and age in each diet group.

Ad Libitum Diet

The effect of parasite load and male age on the total duration of 9 courtship displays are shown in Table 7. None of the courtship display durations were affected by total parasite load. However, the duration of juddering and the duration of raising wings were both significantly affected by the age of the male. The mean juddering duration of older males was significantly longer (13.0 ± 11.9 s) than for young males (6.1

Table 7. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the total duration of 9 courtship behaviour displays in Ad Libitum diet males. Parasite load and age were grouped into three categories (low, medium, high), N=51.

Display	$\bar{x}$ ± s.d. (s)	Parasite F	Parasite p	Age F	Age p	Interaction F	Interaction p
antennate	26 ± 24	1.17	0.32	0.93	0.41	1.21	0.32
turn	2 ± 2	1.24	0.30	0.22	0.81	2.44	0.06
judder	10 ± 11	0.49	0.62	3.42	0.04	0.68	0.61
stridulate	18 ± 42	0.56	0.57	0.56	0.58	0.33	0.86
pause	108 ± 162	1.76	0.18	0.81	0.45	1.88	0.13
chase	8 ± 14	0.66	0.08	0.08	0.93	1.68	0.17
shake antennae	3 ± 5	2.75	0.08	2.59	0.09	1.11	0.36
raise wings	11 ± 14	0.16	0.85	3.31	0.05	0.92	0.46
spermatophore transfer	28 ± 8	0.20	0.82	0.35	0.71	0.37	0.83

± 10.1 s). There was no difference in mean duration of juddering between medium age males (8.5 ± 9.3 s) and the other two age groups. The mean time that males spent raising wings was 4 ± 6 s for the young group, 15 ± 16 s for the medium group, and 12 ± 14 s for the old group. Young males raised their tegmina for less time than both the medium aged males ($p=0.01$) and the old males ($p=0.05$).

Nutritionally Deprived Diet

The mean parasite load for the ND diet males was 45 ± 104 ($n=44$) with a range of 0 to 470 parasites. The effect of parasite load on the total duration of 9 courtship displays was analyzed for the ND diet males and the results are summarized in Table 8. None of the durations of the 9 courtship displays were affected by total parasite load.

Percent Duration of Courtship Displays

Since the courtship time was highly variable among males, the data were reanalysed to examine the proportion of time spent performing each display, rather than total duration of each display in seconds. The proportion of time spent performing each display was highly variable among males (Appendix C, Figures iii, iv) and was analyzed with respect to total parasite load and age for both diet groups.

Ad Libitum Diet

A summary of the analysis on percent duration of display as a function of courtship duration in the AL diet males is summarized in Table 9. None of the percent durations of courtship displays were affected by total parasite load. However, the proportion of courtship time devoted to juddering and shaking antennae did vary with male age. Multiple comparisons across age groups indicated that older males spent proportionately more time juddering (4 ± 3%) and shaking

Table 8. Results from Spearman Rank correlations of parasite load and total duration of courtship behaviour displays in Nutritionally Deprived diet males, N=44.

Display	$\bar{x}$ ± s.d. (s)	r_s	p
antennate	22 ± 21	-0.20	0.20
turn	2 ± 2	-0.70	0.63
judder	6 ± 10	-0.20	0.11
stridulate	24 ± 51	0.01	0.96
pause	43 ± 70	0.05	0.75
chase	7 ± 16	-0.03	0.82
shake antennae	1 ± 1	-0.06	0.71
raise wings	19 ± 73	-0.02	0.87
spermatophore transfer	17 ± 14	-0.10	0.34

Table 9. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the percent duration of courtship behaviour displays in Ad Libitum diet males. Parasite load and age were grouped into three categories (low, medium, high), N=51.

Display	$\bar{x}$ ± s.d. (%)	Parasite		Age		Interaction	
		F	p	F	p	F	p
antennate	9 ± 6	0.63	0.54	2.47	0.10	0.28	0.89
turn	1 ± 1	0.42	0.66	1.06	0.36	0.93	0.46
judder	3 ± 3	0.55	0.58	7.36	0.003	0.57	0.69
stridulate	4 ± 7	0.43	0.65	0.44	0.64	0.22	0.93
pause	21 ± 22	2.09	0.14	1.27	0.29	1.83	0.14
chase	2 ± 3	2.44	0.10	0.01	0.99	1.21	0.32
shake antennae	1 ± 3	1.96	0.15	4.54	0.02	0.74	0.57
raise wings	4 ± 4	0.21	0.81	2.89	0.07	0.37	0.83
spermatophore transfer	11 ± 8	1.49	0.24	0.59	0.57	0.98	0.43

antennae (2 ± 3%) than either of the other two age groups. There was no significant difference between young and medium aged males in the proportion of time spent juddering (young 1 ± 2%, medium 2 ± 2%) or shaking antennae (young 0.2 ± 0.2%, medium 1 ± 3%) during courtship.

Nutritionally Deprived Diet

A summary of the analysis on percent duration of display as a function of courtship duration in the ND diet males is summarized in Table 10. The percentage of time a male spent juddering (5 ± 5%) was significantly correlated with total parasite load. Males spent proportionately less time juddering during courtship as parasite load increased.

POST-COPULATORY GUARDING BEHAVIOUR

Total Duration of Guarding Displays

Post-copulatory guarding behaviour can be divided into 8 distinct guarding displays. The total time spent performing each display was variable among males (Appendix C, Figures v, vi). The total time spent performing each display was analyzed with respect to total parasite load in each diet group..

Ad Libitum Diet

The total durations of 8 guarding displays were analyzed for the effects of parasite load and age in AL diet males. Table 11 summarizes the results for the 51 males. None of the guarding display durations were affected by parasite load. However, the age of the male significantly affected the total duration of guard turning. The mean guard turning duration of males in the young age group was 5 ± 5 seconds (n=13), in the medium age group 3 ± 2 seconds (n=18), and in the old age group 4 ± 3 seconds (n=20). The guard turn duration for young males was longer than for medium males but was not

Table 10. Results from Spearman Rank correlations on parasite load and percent duration of courtship behaviour displays in Nutritionally Deprived diet males, N=44.

Display	$\bar{x}$ ± s.d. (%)	r_s	p
antennate	19 ± 13	-0.30	0.06
turn	1 ± 1	-0.06	0.69
judder	5 ± 5	-0.30	0.03
stridulate	13 ± 13	-0.006	0.97
pause	22 ± 21	0.08	0.61
chase	3 ± 8	-0.08	0.61
shake antennae	1 ± 2	0.02	0.90
raise wings	48 ± 263	-0.10	0.53
spermatophore transfer	32 ± 21	-0.005	0.97

Table 11. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the total duration of 8 guarding behaviour displays in Ad Libitum diet males. Parasite load and age were grouped into three categories (low, medium, high), N=51.

Guarding Display	$\bar{x}$ ± s.d. (s)	Parasite F	Parasite p	Age F	Age p	Interaction F	Interaction p
g.antennate	129 ± 120	0.45	0.64	0.22	0.81	0.74	0.57
g.turn	4 ± 3	2.07	0.14	5.33	0.01	2.54	0.05
g.judder	17 ± 15	0.01	0.99	0.94	0.40	1.43	0.24
g.groom	24 ± 27	0.14	0.87	0.40	0.68	0.73	0.58
g.pause	371 ± 114	0.58	0.56	0.42	0.66	0.57	0.69
g.chase	14 ± 22	0.02	0.98	0.60	0.55	0.19	0.94
g.shake antennae	27 ± 21	0.39	0.68	2.41	0.10	0.33	0.86
g.raise wings	2 ± 7	0.17	0.84	0.75	0.48	0.22	0.93

significantly different than for older males. The medium males guard turned for as long as the old males but for a shorter time than the young males. The interaction between parasite load and age on duration of guard turning was also significant. However, later post-hoc multiple comparison tests failed to determine any significant differences between any pair of means.

Nutritionally Deprived Diet

The total duration of 8 guarding displays were analyzed for the effect of parasite load in ND diet males. Table 12 lists the results from a series of Spearman Rank correlations indicating mean durations, r_s values, and p values. None of the duration of guarding displays was significantly correlated with parasite load.

Table 12. Results from Spearman Rank correlations on parasite load and total duration of 8 guarding behaviour displays in Nutritionally Deprived diet males, N=44.

Display	$\bar{x}$ ± s.d. (s)	r_s	p
g.antennate	214 ± 133	-0.05	0.76
g.turn	6 ± 4	-0.01	0.97
g.judder	17 ± 14	0.06	0.68
g.groom	21 ± 57	0.04	0.82
g.pause	294 ± 123	0.07	0.66
g.chase	22 ± 19	0.10	0.41
g.shake antennae	23 ± 19	0.08	0.59
g.raise wings	2 ± 9	0.20	0.18

DISCUSSION

Parasites may play an important role in sexual selection if females choose their mates based on characteristics which indicate overall fitness, vigour, and ability to resist disease (Zahavi 1975, Hamilton and Zuk 1982, Maynard Smith 1985). Females may also choose males based on characteristics which indicate age (Manning 1985). Females should choose to mate with older males since they have proven their ability to survive and therefore may possess superior genes (Weatherhead 1984). Many studies have been conducted on a variety of animal species to test some predictions of these hypotheses (Møller 1990 a, b, Read and Weary 1990, Simmons 1990). In this section, the results from the current study are discussed considering parasite load and age as important factors contributing to variations in calling song and mating behaviour.

VARIATION IN CALLING SONG

Much variation was found in all of the song parameters measured namely, pulse rate, pulse width, burst duration, pulses per burst, interburst interval, and percent missing pulses. Variation in *G. integer* calling song parameters has also been noted by Souroukis *et al.* (1992).

Effect of Parasites

Some of the variation in song parameters may be accounted for by differences in parasite load among males. If parasite load affects the structure of the calling song, then females may be able to discriminate among males by assessing these differences. However, in the current study, none of the variation in 6 song parameters could be attributed to parasite load for either males fed on an *ad libitum* diet, or in the case where males were nutritionally stressed. Simmons and Zuk (1992) similarly found that gregarine infections did not influence any of 15

calling song characteristics measured in the European field cricket *G. bimaculatus*. Although it was not measured in this experiment, parasites might instead influence the duration of song output or the amount of time males sing either nightly or over their entire lives. If the parasites decrease host fitness, the output of calling should be reduced in heavily infected males. Males who have reduced calling will ultimately suffer a decrease in reproductive success. Zuk (1987 c), however, in a study on *G. veletis* and *G. pennsylvanicus*, found that levels of gregarine infections did not influence either the calling duration of males or the number of females attracted to males in experimental arenas.

In this study, gregarines did not affect any of the calling song characteristics. If females can benefit from choosing males for parasite resistance, why don't males advertize in their call that they are resistant to parasites? It may be that calling song is simply not a reliable signal to assess parasite resistance. One condition of the Hamilton and Zuk (1982) hypothesis, and of sexually selected traits in general, is that they should be costly for individual males to produce and maintain. This costliness is essential to maintain the honesty of the advertisement (Kodric-Brown and Brown 1984). However, if a signal is not costly, there may be "cheaters" (Williams 1966). In this case, the cheaters may be males who are not resistant to parasites, but who are able to produce songs that sound like the songs of resistant males. If this happens, the signal or song could no longer be used by females as a reliable indicator of male parasite resistance. While calling is an expensive behaviour (Prestwich and Walker 1981), perhaps maintaining the pattern within the song is not. Therefore, there would

be no selection on males to advertise parasite resistance through variation in the fine stucture of calling song.

Effect of Age

Another source of variation in calling song parameters might be attributed to differences in male age. In this study, however, age could not account for any of the variation in song parameters in an *ad libitum* diet group. Souroukis *et al.* (1992) also found that age had no effect on calling song parameters of *G. integer*. Similarly, Simmons and Zuk (1992) studied the calling song of *G. bimaculatus* and found that none of the song parameters measured were correlated with male age. Other workers have examined the relationship between measurements of song such as bout length and calling duration and male age, in the California field cricket *G. integer* (Hedrick 1986) and the Texas field cricket *G. integer* (Cade and Wyatt 1984), but no positive correlation was found in either case.

Females have been shown to pair preferentially with older males in several species (Zuk 1987 a, 1988 for *G. pennsylvanicus* and *G. veletis*, Simmons and Zuk 1992 for *G. bimaculatus*), however, no characteristics in the call have been found to correlate with male age. If females tend to prefer to mate with older males, why does it seem that older males are not advertising their age in their calls to females? Again, such truth in advertising only pays if cheating is impossible. It has been proposed that age is a difficult "trait" to falsify and therefore should be an important and reliable cue for females (Kodric-Brown and Brown 1984). The idea of cheaters invading the system is difficult to explain if age is hard to fake, such as in cases where changes in the signal are dependent on physiological changes which come about only as males age.

Acoustic signals of other animals appear to contain information about male age. For example, male stoneflies, *Pteronarcella badia*, attract females by drumming their abdomen on the substratum. Zeigler and Stewart (1985) found that younger males drummed longer than older males. In at least one species of frog, *Physalaemus pustulosus*, differences in male calls are probably age-related, and females prefer the calls of older males (Ryan 1980). However, in this species, age is correlated with size, so females choosing larger males are also choosing older males. In crickets, size in not an indicator of male age since crickets do not grow past their imaginal moult. Females appear to prefer older males (Zuk 1987 c), but the results from this and other studies (Cade and Wyatt 1984, Hedrick 1986, Zuk 1987 c, Simmons and Zuk 1992) indicate that information on male age is not contained in the calling song structure.

VARIATION IN COURTSHIP BEHAVIOUR

Females may make more specific mate choices once they are attracted to a calling male and are in close contact with the male (Zuk 1987 a). Such close contact occurs during courtship. Courtship might be energetically expensive for males. It is possible that variation in male courtship behaviour can be attributed to several factors, including parasite load and age.

Mating Success and Courtship Duration

Effect of Parasites

If parasites affect the fitness of males, it is expected that heavily parasitized males should have reduced mating success. In this study, gregarine parasite load did not affect whether a male mated or not in either diet group. Males were equally likely to mate during the mating trials irrespective of whether they were parasitized or not. It has been

proposed that in animals where females require precopulatory courtship, it is likely that males that initiate courtship quickly and court actively will be more successful in obtaining mates (Woodhead 1986). It might be expected, therefore, that males with fewer parasites would take less time to initiate courtship, complete courtship, and mate than heavily infected males. In this study, however, parasite load did not affect the time that a male was with a female before achieving a mating, the latency to courtship, or the duration of courtship.

A previous study has shown that, under certain circumstances, parasites can affect the mating success of males. Simmons (1993) found that male bushcrickets, *Requena verticalis*, that were heavily infected with gregarine parasites mated less frequently than less infected males, but only when males were fed a low protien diet. This indicates that diet may be important in determining how parasites affect fitness. Simmons suggested that males fed on a protein-deficient diet were unable to overcome the constraints imposed by parasitic infections. Yet, in the present study, parasites did not affect the mating success of males on a nutritionally deprived diet. Perhaps these males still received sufficient nutrition prior to the mating trials. The males in this study had been fed an *ad libitum* diet as nymphs and as adults up to 10 days of age, and were then starved for five days. The high protein diet during nymphal and early adult development may have been sufficient to build adequate resources to give even a starved male enough energy to successfully resist an infection. Perhaps if the nutritionally deprived males had been reared on a poor diet for their entire lives, the less resistant males would have had a more difficult time fighting parasite infections.

It should be noted in this study that mating success in the present study was determined by observing a single mating. If mating behaviour had been observed over a longer period of time, the effect of parasites may have been seen. Infected males may not have been able to produce spermatophores as quickly as resistant males and this would have reduced the lifetime reproductive success of males. Zuk (1987 c) studied the effect of gregarines on spermatophore production in *G. pennsylvanicus* and found that spermatophore production was reduced from 4-8 spermatophores in uninfected males, to 0-8 in infected males over a 24 hour period. However, Souroukis and Cade (1993) found that the mating success for *G. pennsylvanicus* males ranges from 0-6 matings per night. Therefore, the reduction in spermatophore production in infected males may not substantially affect their mating success. Males seem to need to produce only a few spermatophores per night which even heavily infected males would be able to produce. However, Zuk (1987 c) argued that often two or more females are attracted to the same male and that males must be able to provide spermatophores to all females to achieve maximum mating success. Perhaps in cases were multiple females are with a single male, the negative effect of gregarines on spermatophore production is more important in influencing male reproductive success. However, in circumstances where a single male is with a single female, reduction in spermatophore production from gregarine infections shouldn't affect male mating success. The effect of the gregarines may only be noticable after a male had been with a female for longer that 2 hours (Zuk 1987 c).

It is also important to note that female crickets observed in the present courtship experiments were relatively old virgins (15 - 25

days) and were probably highly motivated to mate. Such older females have a lower reproductive value and therefore may increase their reproductive effort (Stearns 1992) and be less choosy about the quality of their mate and not discriminate for parasite resistance .

The effect that starvation of the host has on the gregarines is not known but may be important in determining infection levels. If there is no food in the gut of the host, gregarines should also suffer. In such a situation, parasites might form cysts and leave the host, so that nutritionally depriving males may have the effect of clearing the host of parasites.

Effect of Age

Age may affect mating success. Older males have lower residual reproductive value and thus may exhibit increased reproductive effort (Stearns 1992). Older males may attract more females if they carry higher fitness genes (Manning 1985). In this study, there was no effect of age on male mating success. Females tended to mate equally with older and younger males.

Zuk (1987 c) found that age was important in determining mate attraction in an arena experiment where male *G. veletis* and *G. pennsylvanicus* of different ages were suspended over pitfall traps. Females were more likely to approach older than younger males. However, whatever cues the females were using to discriminate among males was not determined. Similarly, female fruit flies, *Drosophila melanogaster*, mated more frequently with old males than with young males when they were given a choice (Long *et al.* 1980). However, it was not determined if females mated with older males because they preferred them, or because older males were more successful in male-male competition.

It was also found in the present study that the time to achieve a mating, the latency to courtship, and the courtship duration were not affected by age. Long *et al.* (1980) also found no differences in courtship initiation, courtship duration, or total time spent courting between young and old male *D. melanogaster*. In contrast, Woodhead (1986) studied the effect of male age on mating success in the cockroach, *Diploptera punctata*, and found that young males were slower to initiate courtship than older males, and that females accepted courtship from older males more frequently than from younger males. Male *D. punctata* transfer nutritious material to the female during mating and accumulate this material if they do not have the opportunity to mate which causes older males to have more sperm and larger spermatophores than young males. It was suggested that it may not be male age, but the amount of reproductive material that males transfer which affects female choice.

Interaction Between Parasites and Age

A significant interaction was found between parasite load and male age with respect to courtship duration. It might be expected that only those males who are both very old and heavily parasitized would be in such poor condition that courtship duration would be negatively affected. Unfortunately, multiple post-hoc comparison tests failed to detect significant differences between any pair of means. This failure probably reflects the fact that the analysis of variance is a more powerful test than the multiple comparison test (Zar 1982), and that some of the sample sizes were very small. For example, only 2 males were represented in the very old and highly parasitized category. Larger sample sizes in each cell might determine if this interaction really exists.

Courtship Displays

Although having parasites or being old did not affect a male's probability of mating during the experimental trials in this study, it might still be predicted that courtship displays may vary among males with respect to either parasite load or age.

Effect of Parasites

Males that are resistant to parasites are expected to be more vigourous than infected males and therefore are expected to have longer and more elaborate courtship displays (Hamilton and Zuk 1982, Maynard Smith 1987). The effect of gregarine parasites on the total duration of 9 courtship displays was studied. There was great variation among males in the total duration of all 9 courtship displays. Similarly, Boake (1984) found high variability for 8 courtship displays observed in a gregarious cricket, *Amphiacusta maya*. In this study, none of the total courtship display durations were affected by total parasite load in either diet group.

The amount of time a male spent performing pre-copulatory behaviour varied greatly from 30 s to 15 min. Because of this variability, perhaps females judge male quality by evaluating the proportion of time males spend performing courtship displays instead of total time. The displays that are most energetically expensive might be used as cues by females to assess male vigour. Parasitized males would be expected to perform energy-demanding courtship displays for proportionately less time than unparasitized males. In this study, there was great variation in the proportion of time males spent performing the 9 different courtship displays. In the *ad libitum* diet group, there was no relationship between parasite load and the proportion of time males spent performing the displays. However, the nutritionally

deprived males did show significant differences in behaviour with respect to parasite load. Males in the nutritionally deprived diet group spent proportionately less time juddering during courtship. Juddering might be energetically expensive since the male vigourously shakes his body backwards and forwards which seems to utilize many muscles. Such displays may only be affected by parasite load when males are food-stressed. Such stress may occur in natural environments during times of food shortage. Only the most fit males would be able to withstand nutritional deprivation and still perform energetically expensive juddering displays for long durations. Variation in juddering might therefore be used by females to assess male fitness but would only be reliable under certain environmental conditions.

Effect of Age

Older males have lower residual reproductive value (Stearns 1992), and therefore should put maximum energy into present reproductive effort (Manning 1985). This increase in reproductive effort could be translated into longer display durations for older males. The proportion of time spent performing particular courtship displays may also change as males age (Long *et al.* 1980). In the *ad libitum* diet group, where males were various ages, older males juddered and raised their wings for a longer period of time and also juddered and shook their antennae for proportionately longer than younger males. These findings support the predictions.

Juddering and shaking antennae may be constrained by energy resources as both seem quite vigorous (personal observations). The display of males raising their wings, without producing courtship song, seems unclear. Males often performed this behaviour for several seconds before producing actual song. This silent behaviour has not

been reported in other descriptions of *Gryllus* courtship. In this study, as the age of the males increased, they spent proportionately more time juddering and shaking their antennae during courtship. Antennal shaking also appears to be energetically expensive since it involves vigorous and often lengthy shaking of both antennae simultaneously. The theory that older males will invest more energy into more energetically expensive displays is supported.

VARIATION IN GUARDING BEHAVIOUR

Male *G. integer* perform post-copulatory mate guarding. This is thought to prevent females from removing spermatophores before they have been emptied of sperm (Sakaluk 1984, 1985). By prematurely removing spermatophores, females can alter the paternity of their offspring (Simmons 1990). Males differ in how they perform guarding behaviour and the variability may be due to differences in male quality. Guarding behaviour of males may be evaluated by females as an indicator of male viability (Sakaluk 1984, 1985). Great variation was found in the durations of 8 guarding behaviour displays for male *G. integer* in this study.

Effect of Parasites

If parasite-free males are more vigourous than males with many parasites, it is expected that parasite free males should be able to guard females more effectively than parasitized males. As parasite load increases, guarding displays should decrease in duration. In this study, none of the guarding display durations were affected by parasite load in either the *ad libitum* or nutritionally deprived diet group.

These results are in contrast to those reported by Simmons (1990) on the European field cricket, *Gryllus bimaculatus*. He observed a single male with a single female in a small arena and observed guarding

behaviour. Active guarding behaviour was measured as the sum of several guarding displays. The duration of active guarding behaviour increased with higher levels of gregarine infections. This is contrary to the prediction that infected males should be less vigourous but Simmons argued that infected males had to guard more intensely to prevent the female from leaving.

Simmons (1990) also conducted a similar study using a much larger arena to simulate a more natural environment where females could leave males after a mating. Again, the time that a male was able to successfully guard a female was measured and correlated with parasite load. The results indicated a negative relationship between parasite load and duration of guarding. Males that were heavily infected guarded females for less time than males with light infections. This suggested heavily infected males were unable to retain females long enough to ensure their fertilization success and was in direct contrast to the findings from the small arena. The contrast in findings highlights the importance of the experimental design when examining the influence of parasites. Simmons assessed parasite load as the number of cysts in the gut only. In fact, results from the present study indicate that there is no clear relationship between number of trophozoites in the gut and number of cysts in the faeces. Therefore, cyst counts alone might not be reliable indicators of a male's parasite burden.

Simmons assessed parasite load as the number of cysts in the gut, whereas this study included trophozoites in the gut and cysts in the faeces as well. Also, all of the "active" guarding display durations (antennate, judder, and antennal shaking) were added together in Simmons' study and correlated with total number of cysts. In this

study, each display duration was correlated with parasite load and none of the separate behaviours were affected by parasite load. The measurement of parasite load in this study was more accurate than the method used by Simmons of only counting cysts in the gut. These differences should caution researchers not to make general assumptions about the affects of gregarine infections on the guarding behaviour of male crickets.

Effect of Age

In this study, only one post-mating behaviour was affected by male age and that was the amount of time males spent turning during guarding behaviour. Older males guard turned for shorter durations than younger males. The prediction that older males should perform the displays for longer durations was not upheld by this finding. It is not understood why older males should perform a display for shorter durations. Turning includes turning both towards and away from the female. This usually happens when the male is re-establishing contact with the female or is performing other displays and periodically turns to antennate the female. An increase in turning display therefore indicates that young males probably had to regain contact with the female more often than older males for unknown reasons.

Interaction Between Parasites and Age

For one of the guarding behaviours, there was a significant interaction between parasite load and age. The interaction between parasite load and age on the length of time a male spent turning either towards or away from a female during guarding behaviour was significant. It may be expected that only those males who are very fit, by being both old and parasite resistant, are in such good condition that

they would only have to turn towards and away from the female for short durations to keep her guarded successfully. However, multiple post-hoc comparison tests, being less rigourous than the initial ANOVA test, again failed to detect differences between any pair of means (Zar 1982) and the predictions can only be tested effectively with higher sample sizes.

CONCLUSIONS

Males of many species often vary in the expression of secondary sexual traits. If the expression of these traits is a reflection of a males viability, females who express a preference for elaborate traits and displays may benefit by mating with those males who are fit as reflected in the quality of these traits. These females might gain heritable genetic benefits for their offspring (Hamilton and Zuk 1982, Manning 1985).

Several studies on various orders of animals have investigated the possibility that females make choices based on the quality of a male's traits. Specifically, females may choose mates for traits which indicate parasite resistance (Hamilton and Zuk 1982). The results from these studies have been equivocal. Some studies support the hypothesis, while others do not. The current study found very little evidence to support the theory that variation in male calling song, courtship displays, or guarding displays can be correlated with parasite load. If this is so, then females cannot gain reliable information on male resistance from assessing variations in these behaviours.

In only a few cases (Zuk 1987 a,b, Simmons 1990, Simmons and Zuk 1992, Simmons 1993) have significant relationships between parasites and behaviours in other orthopteran species been reported, and even these have been ambiguous. High parasite loads might

decrease spermatophore production (Zuk 1987 b), but not enough to a biologically significant extent. Studies performed by Simmons (1990, 1993) suggested that while parasites may be significant in some circumstances, in others they may have little relevance depending on the species, environment, or dietary conditions.

It has been proposed for mate choice based on parasite resistance to evolve, that the prevalence and abundance of the parasite should be high in the host population, and the parasites should be as little aggregated as possible among their hosts. Only under these conditions can the choice for vigourous males remain adaptive even when some susceptable males remain uninfected because they are not exposed to the parasite (Poulin and Vickery 1993). If there is great variation in parasite prevalence between seasons and years, and if parasite intensity is highly variable, parasite resistance becomes an unreliable trait to assess male fitness (Weatherhead *et al.* 1992). Information from the present study indicated that up to 50% of laboratory males were uninfected by gregarine parasites, with only a few males having very high loads. Information from preliminary sampling of natural levels of parasite infection from field population studies (MacDougall and Murray, unpublished data) seem to indicate similar aggregations of parasite levels as in lab populations. These characteristics of gregarine infections suggest they may not be an effective selective force in the evolution of female preference for unparasitized males.

The results from this study also indicate that, in a few cases, variation in male behaviour was associated with male age. A few other studies have suggested that age is important in Orthopteran mating success (Zuk 1987 c, Simmons and Zuk 1992), but none have correlated any variations in male secondary sexual traits with age. The traits

found to be correlated with age in this study may be important if females assess male displays for old age as indicators of superior genes.

It could be argued that in animals who undergo great stresses in their juvenile stages of life, males may have already shown that they possess great survival ability by reaching adulthood (Tinsley 1990). By surviving several moults and avoiding predators and diseases, by making it to adulthood, each adult cricket may have proven itself to have superior genes simply by surviving.

Although several studies have supported the idea that variations in male behaviour may be attributed to differences in parasite load, gregarine gut parasites did not affect the calling song characteristics or mating behaviour of *G. integer*. This implies that gregarines do not play an important role in sexual selection in this species. This host-parasite system may not be a suitable one for testing the Hamilton and Zuk hypothesis for several reasons already suggested. However, since some displays were correlated with male age in this study, it seems that variation in courtship, and perhaps in guarding behaviour, may indicate differences in male age and therefore be an important factor influencing female choice.

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Appendices

Appendix A

Table i. Raw data from song recordings of Ad Libitum diet males. Age is given in days, weight in milligrams, and morphometric measurements (pronotum length, pronotum width, femur length, femur width) in centimetres. Also given are the song characteristics pulse rate (PR) in number of pulses/second, pulse width (PW), burst duration (BD), and interburst interval (II) in milliseconds. Data on pulses per burst (P/B) and percent missing pulses (%MP) are also presented.

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	PR	PW	BD	P/B	II	%MP
1	0	32	.69	.44	.44	1.25	.64	66	11	335	22	217	5
2	0	15	.39	.35	.35	1.16	.54	61	10	558	34	263	19
3	0	34	.60	.43	.43	1.25	.64	60	16	718	38	254	16
4	0	16	.46	.38	.38	1.12	.57	56	9	555	31	193	23
5	0	24	.44	.35	.35	1.10	.56	48	6	564	26	285	37
6	0	19	.35	.31	.31	1.03	.46	49	7	442	21	216	46
7	1	22	.40	.36	.36	1.03	.54	73	11	340	25	165	8
8	4	25	.61	.41	.41	1.26	.64	67	10	782	57	194	6
9	4	37	.54	.40	.40	1.28	.60	69	10	662	46	225	37
10	249	23	.68	.40	.40	1.35	.63	67	6	465	31	330	16
11	3	15	.43	.36	.36	1.12	.53	52	7	297	15	414	33
12	0	34	.51	.36	.36	1.14	.54	52	8	874	47	325	33
13	5	11	.35	.33	.33	1.00	.49	68	5	226	15	376	13
14	6	25	.55	.39	.39	1.20	.60	60	5	324	20	270	10
15	8	14	.50	.37	.37	1.17	.57	59	8	446	27	353	18
16	49	23	.47	.34	.34	1.15	.55	65	11	568	36	263	10
17	66	20	.26	.31	.31	1.03	.47	58	7	317	19	330	13
18	24	23	.43	.35	.35	1.08	.57	57	9	391	23	324	12
19	78	32	.38	.35	.35	1.27	.55	51	8	224	11	407	26
20	6	14	.39	.33	.33	1.01	.52	58	5	405	23	267	17

Table i. (continued)

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	PR	PW	BD	P/B	II	%MP
21	4	14	.49	.41	.41	1.21	.61	61	10	515	31	264	14
22	0	38	.36	.35	.35	1.05	.53	70	7	387	27	250	4
23	2474	37	.49	.36	.36	1.13	.53	66	5	753	58	165	26
24	0	36	.43	.36	.36	1.08	.54	63	7	333	20	334	20
25	0	27	.43	.36	.36	1.13	.56	58	8	656	36	308	34
26	0	50	.38	.38	.38	1.10	.57	66	8	400	27	264	7
27	0	23	.44	.38	.38	1.14	.59	67	7	504	33	316	10
28	141	40	.45	.30	.36	1.14	.58	66	7	412	27	289	12
29	1221	28	.34	.32	.32	1.03	.50	62	7	272	16	182	25
30	0	22	.38	.37	.37	1.10	.55	66	7	317	21	393	8
31	0	20	.50	.42	.42	1.22	.60	64	9	550	35	223	5
32	0	35	.35	.34	.34	.98	.53	66	7	557	37	216	2
33	7	11	.48	.40	.40	1.07	.60	60	12	360	21	343	13
34	0	18	.52	.40	.40	1.21	.64	48	20	437	7	162	37
35	0	22	.67	.46	.46	1.24	.72	63	7	303	19	289	16
36	35	19	.40	.54	.54	1.06	.37	67	9	492	32	312	17
37	0	14	.41	.38	.38	1.11	.57	66	6	371	24	219	14
38	0	20	.72	.46	.45	1.30	.67	64	9	509	32	206	15
39	3	22	.66	.40	.40	1.21	.62	61	10	392	24	278	18
40	0	29	.62	.43	.43	1.24	.64	71	9	353	25	227	4
41	40	33	.42	.35	.35	1.06	.55	58	7	416	23	342	14
42	0	16	.57	.39	.39	1.18	.62	69	11	398	27	232	12
43	25	9	.39	.36	.36	1.08	.54	70	6	432	30	210	9
44	0	14	.40	.34	.34	1.08	.55	73	7	361	27	200	6
45	2000	18	.58	.44	.44	1.20	.64	53	4	206	12	284	45
46	173	18	.45	.35	.35	1.11	.57	70	10	408	26	232	9

Table ii. Raw data from song recordings of Nutritionally Deprived diet males. Age is given in days, weight in milligrams, and morphometric measurements (pronotum length, pronotum width, femur length, femur width) in centimetres. Also given are the song characteristics pulse rate (PR) in number of pulses/second, pulse width (PW), burst duration (BD) and interburst interval (II) in milliseconds. Data on pulses per burst (P/B) and percent missing pulses (%MP) are also presented.

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	PR	PW	BD	P/B	II	%MP
1	470	15	.50	.42	.65	1.22	.43	64	4	460	28	370	15
2	20	15	.45	.38	.59	1.21	.40	67	8	672	46	320	3
3	28	15	.34	.39	.58	1.08	.40	68	7	341	22	331	11
4	0	15	.31	.30	.51	1.00	.34	66	5	229	15	291	11
5	0	15	.23	.27	.45	.88	.30	72	8	274	20	274	13
6	18	15	.49	.41	.61	1.19	.40	65	9	249	16	213	19
7	183	15	.28	.31	.49	1.00	.36	69	8	383	21	335	10
8	13	15	.35	.35	.52	1.01	.36	72	7	420	30	382	8
9	9	15	.53	.42	.62	1.24	.41	66	9	422	28	283	15
10	8	15	.49	.40	.58	1.16	.39	66	10	456	29	263	16
11	5	15	.53	.43	.59	1.22	.44	62	12	525	30	294	19
12	60	15	.58	.43	.67	1.19	.41	60	7	973	68	389	13

Table iii. Raw data from mating behaviour experiments of Ad Libitum diet males. Age is given in days, weight in milligrams, and morphometric measurements (pronotum length, pronotum width, femur length, femur width) in centimetres. Data on the 9 courtship displays are given in seconds and in percent (proportion of courtship time spent performing each display). The data from the 8 guarding (g.) displays, time to mate, latency to courtship, and courtship duration are also presented in seconds.

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	antennate	turn	judder	stridulate	pause	chase
1	0	16	.52	.42	.63	1.20	.44	7.4	2.4	2.4	11.9	8.3	0
2	0	34	.52	.41	.62	1.20	.42	12.9	7.9	24.3	27.5	42.1	2.0
3	1002	23	.49	.40	.61	1.21	.44	14.8	1.8	9.1	6.1	86.9	37.0
4	42	22	.49	.39	.57	1.15	.41	52.1	5.3	25.0	2.4	234.2	30.9
5	25	21	.60	.44	.64	1.25	.43	12.2	.4	.6	9.2	0	0
6	1511	17	.56	.41	.61	1.24	.38	13.5	6.1	7.4	1.4	525.2	46.8
7	159	21	.64	.42	.62	1.27	.43	8.3	1.2	3.7	1.6	0	0
8	298	26	.61	.44	.65	1.28	.41	15.5	2.2	2.8	2.0	47.9	0
9	0	12	.50	.39	.59	1.17	.40	30.5	2.5	0	11.2	136.4	12.9
10	930	24	.38	.35	.55	1.07	.36	14.9	.7	2.6	1.1	10.6	0
11	738	21	.42	.37	.55	1.10	.35	7.3	1.5	7.7	13.1	11.4	0
12	84	24	.23	.23	.40	.85	.26	35.3	12.1	30.6	223.8	199.2	56.4
13	1	26	.56	.40	.62	1.16	.42	140.0	8.0	18.0	195.8	176.9	1.5
14	1	26	.59	.41	.62	1.23	.41	51.0	1.9	10.4	1.8	137.6	28.1
15	128	23	.45	.39	.59	1.26	.43	34.2	4.5	13.9	97.6	349.3	20.0
16	1	23	.58	.37	.63	1.26	.44	46.2	2.0	0	22.6	788.8	4.6
17	20	19	.57	.39	.59	1.23	.44	5.7	.6	1.4	5.4	.1	0
18	16	15	.51	.37	.59	1.14	.38	11.8	1.6	37.6	9.0	183.5	4.8
19	15	19	.55	.39	.59	1.20	.40	25.6	1.5	11.8	0	175.2	2.7
20	1140	24	.61	.43	.64	1.19	.42	89.5	4.7	25.7	47.8	34.2	1.6

Table iii. (continued)

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	antennate	turn	judder	stridulate	pause	chase
21	0	18	.46	.39	.58	1.12	.39	4.0	3.3	0	17.7	24.6	14.3
22	179	20	.68	.39	.69	1.26	.44	11.4	.7	3.0	4.3	32.3	0
23	0	18	.68	.37	.62	1.19	.39	9.4	0	6.4	.6	7.0	0
24	639	24	.61	.45	.67	1.27	.45	5.8	.8	8.8	4.3	5.0	.4
25	20	23	.50	.44	.64	1.22	.41	46.4	.7	4.2	3.5	107.8	21.7
26	0	23	.51	.40	.60	1.23	.40	15.6	.7	3.9	12.8	0	0
27	1416	28	.49	.61	.37	1.17	.39	13.4	.7	3.4	1.2	18.2	0
28	278	21	.46	.37	.57	1.08	.38	14.5	1.2	2.5	3.2	0	0
29	225	33	.57	.42	.60	1.19	.40	7.7	.8	1.5	1.0	25.3	.7
30	337	34	.51	.41	.62	1.19	.39	22.0	.7	8.9	8.6	0	0
31	152	26	.42	.38	.54	1.10	.37	24.6	.7	23.4	4.4	55.0	0
32	2003	30	.51	.38	.62	1.21	.40	33.7	0	6.2	2.6	26.5	0
33	1	30	.29	.31	.47	.99	.32	38.8	1.4	11.9	7.3	19.9	0
34	584	22	.55	.40	.61	1.19	.40	12.8	0	.6	0	40.4	0
35	1	23	.42	.33	.56	1.08	.36	45.0	.7	3.3	12.7	106.1	2.5
36	637	14	.49	.39	.58	1.15	.40	33.0	2.7	0	34.2	452.3	13.1
37	4	32	.40	.41	.63	1.21	.41	5.1	1.6	9.5	1.0	52.4	4.1
38	475	42	.51	.41	.63	1.21	.41	13.5	0	2.4	.9	34.9	6.4
39	1031	30	.47	.38	.58	1.13	.37	65.8	2.6	18.7	2.8	178.1	18.6
40	807	30	.57	.39	.60	1.17	.39	30.2	5.2	41.0	0	140.1	3.5
41	9	13	.38	.34	.54	1.05	.36	21.7	.7	6.9	0	269.0	1.8
42	1186	30	.53	.39	.61	1.23	.40	10.9	1.3	8.8	21.0	31.5	19.5
43	68	28	.50	.38	.57	1.16	.41	50.0	4.7	42.6	6.1	340.7	42.6
44	459	27	.54	.37	.57	1.17	.40	9.1	.6	5.0	6.7	1.9	0
45	975	24	.70	.44	.68	1.25	.44	1.0	.6	4.4	7.8	11.8	10.3
46	230	27	.67	.45	.65	1.28	.44	33.4	1.2	6.9	0	4.5	0
47	665	32	.40	.37	.56	1.16	.41	24.5	1.6	5.2	35.6	20.0	0
48	3	15	.45	.35	.57	1.14	.38	8.3	2.1	2.6	22.3	37.2	1.0
49	1184	25	.37	.35	.55	1.06	.36	42.1	.5	6.7	2.0	120.7	0
50	66	15	.47	.41	.59	1.18	.42	17.3	.9	0	3.3	0	0
51	156	34	.54	.43	.63	1.26	.42	11.5	1.4	9.7	3.7	0	0

Table iii. (continued)

	shake antennae	raise wings	sperm.transfer	%antennate	%turn	%judder	%stridulate	%pause	%chase	%shake antennae	%raise wings
1	1.2	9.9	51.2	4.3	1.4	1.4	6.9	4.8	0	.7	5.7
2	0	7.0	18.2	3.8	2.3	7.2	8.1	12.5	.6	0	2.1
3	1.2	8.2	22.7	5.7	.7	3.5	2.3	33.3	14.2	.5	3.1
4	0	31.1	26.6	8.5	.9	4.1	.4	38.3	5.0	0	5.1
5	0	.9	32.2	10.6	.3	.5	8.0	0	0	0	.8
6	2.0	.5	28.6	1.8	.8	1.0	.2	71.9	6.4	.3	.1
7	0	14.5	26.6	2.1	.3	.9	.4	0	0	0	3.7
8	0	8.8	31.1	6.3	.9	1.1	.8	19.4	0	0	3.6
9	0	1.7	27.1	9.4	.8	0	3.4	42.0	4.0	0	.5
10	14.0	14.5	26.3	12.6	.6	2.2	.9	9.0	0	11.9	12.3
11	3.2	8.5	30.0	2.3	.5	2.5	4.2	3.7	0	1.0	2.7
12	1.0	67.1	33.4	6.0	2.0	5.2	37.8	33.6	9.5	.2	11.3
13	9.3	55.3	32.9	21.5	1.2	2.8	30.0	27.1	.2	1.4	8.5
14	22.4	6.8	31.5	14.3	.5	2.9	.5	38.7	7.9	6.3	1.9
15	5.6	34.7	29.2	3.7	.5	1.5	10.7	38.2	2.2	.6	3.8
16	0	7.8	32.8	3.8	.2	0	1.9	65.5	.4	0	.6
17	0	2.3	24.2	6.8	.7	1.7	6.4	.1	0	0	2.7
18	2.2	8.8	14.8	1.8	.2	5.9	1.4	28.7	.7	.3	1.4
19	1.7	1.6	30.2	4.8	.3	2.2	0	32.6	.5	.3	.3
20	.6	20.7	27.5	18.3	1.0	5.3	9.8	7.0	.3	.1	4.2

Table iii. (continued)

	shake antennae	raise wings	sperm.transfer	%antennate	%turn	%judder	%stridulate	%pause	%chase	%shake antennae	%raise wings
21	0	3.8	25.9	.3	.3	0	1.4	2.0	0	0	3.9
22	0	1.5	29.2	4.2	.3	1.1	1.6	11.9	0	11.4	4.4
23	0	1.1	18.2	1.5	0	1.0	.1	1.1	1.1	0	.3
24	1.1	4.0	38.4	1.1	.2	1.7	.9	1.0	0	0	.6
25	0	3.2	19.5	17.1	.3	1.5	1.3	39.8	0	0	.2
26	.9	2.6	36.9	10.8	.5	2.7	8.8	0	.1	.2	.8
27	1.8	.7	46.9	8.4	.4	2.1	.8	11.4	8.0	0	1.2
28	0	15.1	25.8	18.8	1.6	3.2	4.2	0	0	.6	1.8
29	2.9	15.6	20.3	7.9	.8	1.5	1.0	26.1	0	1.1	.4
30	0	8.2	31.6	9.9	.3	4.0	3.9	0	0	0	19.6
31	11.7	25.2	17.4	7.5	.2	7.1	1.3	16.8	.7	3.0	16.1
32	5.3	1.0	23.3	8.3	0	1.5	.6	6.5	0	0	3.7
33	19.0	11.9	13.8	15.5	.6	4.7	2.9	7.9	0	3.6	7.7
34	6.3	1.0	32.8	2.6	0	.1	0	8.1	0	1.3	.2
35	8.8	5.6	32.6	9.5	.1	.7	2.7	22.5	0	7.6	4.7
36	2.7	20.8	19.1	5.1	.4	0	5.3	69.5	0	1.3	.2
37	2.3	3.9	18.1	3.5	1.1	6.6	.7	36.4	.5	1.9	1.2
38	1.7	4.4	33.6	2.8	0	.5	.2	7.2	2.0	.4	3.2
39	0	3.5	21.4	19.4	.8	5.5	.8	52.4	2.8	1.6	2.7
40	8.0	38.8	33.4	9.6	1.7	13.0	0	44.5	1.3	.3	.9
41	0	0	32.5	6.5	.2	2.1	0	80.5	5.5	0	1.0
42	4.7	11.2	23.3	4.8	.6	3.9	9.3	14.0	1.1	2.5	12.3
43	0	4.1	44.8	8.8	.8	7.5	1.1	60.2	.5	0	0
44	6.5	3.0	29.3	3.2	.2	1.8	2.4	.7	8.7	2.1	5.0
45	2.3	17.2	23.9	.2	.1	.8	1.4	2.1	7.5	0	.7
46	1.3	3.3	41.6	19.8	.7	4.1	0	2.7	0	2.3	1.1
47	1.3	12.3	15.8	10.0	.7	2.1	14.5	8.1	1.8	.4	3.0
48	0	1.6	14.5	2.3	.6	.7	6.1	10.2	0	.8	2.0
49	8.7	12.5	23.0	14.2	.2	2.3	.7	40.8	0	.5	5.0
50	0	3.7	14.7	18.4	1.0	0	3.5	0	.3	0	.4
51	13.3	5.2	24.0	9.8	1.2	8.3	3.2	0	0	2.9	4.2

Table iii. (continued)

	%sperm.transfer	g.antennate	g.turn	g.judder	g.groom	g.pause	g.chase	g.shake antennae	g.raise wings	time to mate	latency	court duration
1	29.6	49.4	3.9	44.8	15.1	325.3	74.4	55.2	0	173	66	107
2	5.4	5.8	6.8	21.8	1.9	436.5	4.4	31.1	31.9	338	120	218
3	8.7	89.6	6.0	49.8	50.4	273.3	67.8	23.5	0	261	45	216
4	4.3	116.2	0	.5	30.7	408.3	0	25.9	0	612	182	430
5	28.0	78.3	3.5	13.9	.1	386.8	84.4	3.9	0	115	58	57
6	3.9	118.5	9.2	32.3	33.9	316.2	33.1	20.2	0	730	69	661
7	6.8	103.4	1.7	7.2	10.1	434.7	2.6	28.0	.7	392	348	54
8	12.6	87.1	.5	12.4	12.6	413.9	53.0	7.8	0	247	130	117
9	8.3	184.9	3.7	11.7	29.6	322.7	13.6	10.6	0	325	98	227
10	22.3	26.0	4.2	26.6	10.1	462.8	11.4	30.8	8.4	118	16	102
11	9.6	72.1	4.0	21.5	32.3	423.0	.8	26.5	0	312	208	104
12	5.6	1.2	0	2.3	75.3	264.7	0	33.5	32.0	592	106	486
13	5.0	51.3	5.0	9.4	18.4	484.1	4.0	8.4	0	652	49	603
14	8.8	227.6	13.2	5.4	52.3	237.6	20.6	14.7	0	356	46	310
15	3.2	75.5	3.5	5.3	12.4	476.6	.3	13.0	0	914	303	611
16	2.7	60.5	1.2	5.3	12.8	493.5	10.6	3.3	0	1204	292	912
17	28.8	127.1	7.4	24.3	54.0	314.8	21.2	34.0	1.0	84	37	47
18	2.3	30.9	5.6	46.2	31.4	457.4	21.7	1.6	0	640	372	278
19	5.6	140.1	5.2	5.7	0	703.4	8.6	21.6	0	537	279	258
20	5.6	341.0	3.4	9.2	32.9	296.6	1.2	10.9	0	488	225	263

Table iii. (continued)

	%sperm.transfer	g.antennate	g.turn	g.judder	g.groom	g.pause	g.chase	g.shake antennae	g.raise wings	time to mate	latency	court duration
21	2.1	94.1	3.8	7.3	88.6	370.2	19.9	0	0	1250	67	103
22	10.7	167.6	8.6	18.1	30.8	369.3	5.0	1.9	0	272	165	227
23	2.9	96.6	4.3	13.2	24.4	443.6	0	11.2	0	620	567	53
24	7.6	80.5	5.7	21.2	34.4	418.6	5.8	15.6	1.4	505	374	69
25	7.2	197.0	1.2	10.9	3.0	351.4	5.9	18.4	0	271	58	213
26	25.4	123.3	2.5	7.6	28.3	377.1	0	43.9	0	145	70	75
27	29.5	89.5	10.3	49.7	14.3	372.8	13.1	26.5	.2	159	66	93
28	33.5	96.8	4.2	20.4	.4	389.8	19.7	27.0	0	77	13	64
29	20.9	11.0	3.9	54.8	18.5	453.4	6.6	32.7	0	97	18	79
30	14.2	133.6	1.9	26.0	1.8	381.5	0	23.8	0	223	157	66
31	5.3	67.2	4.3	7.0	7.1	464.0	6.6	33.8	.6	328	169	159
32	5.7	143.3	6.3	5.8	45.4	326.3	26.9	24.4	0	408	308	100
33	5.5	60.8	1.9	24.9	6.9	458.8	1.6	29.8	0	251	110	141
34	6.6	69.4	2.4	4.0	13.6	466.5	.6	20.2	14.2	496	402	94
35	6.9	328.3	.9	2.7	0	237.4	1.5	21.7	0	472	243	229
36	2.9	94.9	5.2	3.3	0	453.9	1.7	29.8	0	651	61	590
37	12.6	132.4	5.7	23.2	62.2	264.7	15.9	91.4	0	144	42	102
38	6.9	79.8	.5	4.9	15.4	460.5	0	31.9	0	486	384	102
39	6.3	570.4	0	1.3	0	29.2	.2	0	0	340	27	313
40	10.6	41.9	2.3	12.2	39.3	365.6	0	81.8	9.4	315	5	310
41	9.7	105.1	4.7	58.4	22.1	355.2	27.1	8.3	0	334	2	332
42	10.4	220.2	3.5	4.5	11.4	312.9	.8	37.8	0	225	79	146
43	7.9	325.0	1.4	5.8	12.9	220.2	0	70.8	0	566	18	548
44	10.4	269.0	2.9	8.0	26.4	245.5	0	50.3	0	282	218	64
45	4.2	68.3	2.8	21.0	41.8	394.6	4.9	63.6	0	565	477	88
46	24.6	7.6	1.7	4.8	29.2	510.6	21.8	13.1	0	169	75	94
47	6.4	249.7	4.4	14.8	2.0	277.5	1.7	39.1	0	246	1203	123
48	4.0	3.0	1.6	23.5	0	511.2	0	53.3	0	366	265	101
49	7.8	540.0	2.8	4.1	0	47.0	2.0	1.2	0	296	75	221
50	15.6	25.8	1.8	40.4	147.8	264.3	84.2	17.6	0	94	54	40
51	20.5	90.8	4.0	8.0	0	403.8	19.5	64.8	0	117	54	63

Table iv. Raw data from mating behaviour experiments of Nutritionally Deprived diet males. Age is given in days, weight in milligrams, and morphometric measurements (pronotum length, pronotum width, femur length, femur width) in centimetres. Data on the 9 courtship displays are given in seconds and in percent (proportion of courtship time spent performing each display). The data from the 8 guarding (g.) displays, time to mate, latency to courtship, and courtship duration are also presented in seconds.

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	antennate	turn	judder	stridulate	pause	chase
1	7	15	.51	.40	.62	1.19	.43	1.7	0	0	7.7	0	0
2	12	15	.33	.34	.51	1.00	.36	83.5	6.3	15.3	45.5	57.8	7.9
3	36	15	.44	.38	.82	1.14	.39	18.8	5.6	0	78.2	30.9	10.3
4	2	15	.40	.43	.57	1.14	.40	2.0	1.6	0	0	0	0
5	0	15	.47	.41	.62	1.19	.39	3.7	0	2.4	3.9	2.2	2.2
6	1	15	.36	.36	.55	.87	.39	13.0	1.8	8.3	41.9	7.6	13.9
7	0	15	.26	.35	.50	.96	.32	9.7	.9	3.0	.8	9.5	.5
8	48	15	.38	.33	.49	.99	.35	34.2	6.4	2.5	97.0	210.6	7.3
9	120	15	.28	.33	.50	1.05	.34	21.3	1.0	2.6	20.9	41.3	.4
10	470	15	.50	.42	.65	1.22	.43	6.4	1.3	1.9	3.0	8.6	0
11	0	15	.39	.34	.56	1.10	.39	39.4	1.8	18.9	26.6	41.9	0
12	21	15	.38	.37	.52	1.11	.38	10.5	.5	.7	3.0	26.1	2.5
13	12	15	.45	.38	.56	1.15	.41	29.0	.7	4.8	2.7	25.4	1.0
14	1	15	.38	.35	.52	1.05	.36	7.3	1.7	3.9	37.7	4.5	0
15	45	15	.44	.37	.60	1.09	.42	1.9	.7	0	1.7	0	0
16	20	15	.45	.38	.59	1.19	.40	9.6	.6	7.3	2.6	2.2	0
17	7	15	.39	1.35	1.55	2.08	1.39	4.1	.2	.3	4.6	0	0
18	27	15	.33	.33	.51	1.03	.34	5.6	.6	20.4	22.2	92.0	92.0
19	5	15	.43	.39	.59	1.14	.41	2.0	.7	0	10.2	0	0
20	0	15	.27	.32	.49	.96	.34	27.8	.4	8.2	7.3	108.9	14.5

Table iv. (continued)

	parasites	age	weight	pron.length	pron.width	fem.length	fem.width	antennate	turn	judder	stridulate	pause	chase
21	0	15	.39	.38	.58	1.12	.41	17.2	1.2	2.5	1.4	21.0	1.9
22	4	15	.27	.33	.50	.97	.35	15.9	1.4	1.3	7.5	1.9	0
23	0	15	.33	.37	.54	1.09	.36	51.0	11.0	1.6	317.8	235.7	43.1
24	2	15	.45	.27	.45	.86	.28	40.2	.4	7.1	.8	0	.9
25	28	15	.34	.39	.58	1.08	.40	36.9	5.9	6.6	50.4	144.2	25.9
26	0	15	.43	.41	.59	1.19	.40	19.4	.9	14.7	8.1	95.0	.7
27	0	15	.45	.32	.47	.91	.29	11.4	.2	0	4.9	5.3	3.8
28	0	15	.44	.35	.57	1.14	.39	18.0	4.4	12.4	67.8	64.4	19.7
29	320	15	.41	.40	.55	1.10	.38	15.6	.4	5.8	0	27.6	.3
30	2	15	.41	.39	.59	1.15	.41	5.0	.4	1.0	0	3.0	0
31	0	15	.25	.31	.48	.87	.31	27.9	1.9	3.9	0	23.6	.4
32	0	15	.33	.34	.52	1.02	.36	53.2	8.0	59.7	5.1	121.1	3.6
33	331	15	.40	.41	.59	1.17	.39	70.5	4.0	8.6	46.4	329.0	29.3
34	210	15	.29	.31	.52	1.03	.36	10.9	1.0	.9	1.3	51.3	9.7
35	2	15	.25	.30	.48	.96	.32	8.5	0	0	12.4	0	0
36	0	15	.52	.42	.61	1.23	.41	27.9	.4	8.6	3.0	0	1.0
37	8	15	.52	.39	.64	1.24	.43	6.3	0	1.5	30.3	16.2	0
38	243	15	.38	.37	.59	1.10	.38	7.6	.6	1.7	2.2	0	0
39	2	15	.41	.40	.61	1.15	.40	35.8	1.4	9.1	3.0	7.1	3.1
40	1	15	.42	.36	.57	1.14	.41	1.7	.7	7.4	2.4	5.3	0
41	0	15	.31	.31	.50	.99	.33	15.1	.8	1.4	1.5	2.6	0
42	0	15	.33	.33	.51	.95	.34	83.7	4.1	6.4	35.6	9.0	1.1
43	0	15	.31	.36	.51	1.00	.34	15.9	1.4	8.0	16.4	0	0
44	0	15	.23	.27	.45	.88	.30	21.4	1.5	2.7	21.6	42.4	.8

Table iv. (continued)

	shake antennae	raise wings	sperm. transfer	%antennate	%turn	%judder	%stridulate	%pause	%chase	%shake antennae	%raise wings
1	0	46.8	24.7	1.7	0	0	7.9	0	0	0	47.8
2	.5	20.4	16.0	34.9	2.6	6.4	19.0	24.2	3.3	.2	8.5
3	.5	24.1	18.4	9.9	2.9	0	41.2	16.3	5.4	.3	12.7
4	0	0	20.2	7.7	6.2	0	0	0	0	0	0
5	1.6	490.1	16.3	13.2	0	8.6	13.9	7.9	7.9	5.7	1750.4
6	0	18.1	34.2	10.3	1.4	6.6	33.3	6.0	11.0	0	14.4
7	0	13.4	13.9	17.6	1.6	5.5	1.5	17.3	.9	0	24.4
8	1.0	18.2	18.5	8.6	1.6	.6	24.4	52.9	1.8	.3	4.6
9	1.7	13.2	21.8	18.1	.8	2.2	17.7	35.0	.3	1.4	11.2
10	.4	4.0	28.2	12.5	2.5	3.7	5.9	16.9	0	.8	7.8
11	0	26.8	24.6	22.5	1.0	10.8	15.2	23.9	0	0	15.3
12	0	1.6	31.3	13.3	.6	.9	3.8	33.0	3.2	0	2.0
13	1.6	1.1	23.3	31.2	.8	5.2	2.9	27.3	1.1	1.7	1.2
14	0	2.8	32.6	8.2	1.9	4.4	42.4	5.1	0	0	3.1
15	0	.4	21.9	7.3	2.7	0	6.5	0	0	0	1.5
16	0	8.7	15.1	20.9	1.3	15.9	5.7	4.8	0	0	18.9
17	0	3.1	19.4	12.8	.6	.9	14.4	0	0	0	9.7
18	0	7.7	26.3	3.1	.3	11.3	12.3	50.8	50.8	0	4.3
19	0	2.0	37.8	4.1	1.4	0	20.8	0	0	0	4.1
20	4.9	5.0	26.1	13.7	.2	4.0	3.6	53.6	7.1	2.4	2.5

Table iv. (continued)

	shake antennae	raise wings	sperm. transfer	%antennate	%turn	%judder	%stridulate	%pause	%chase	%shake antennae	%raise wings
21	1.4	2.9	30.1	22.1	1.5	3.2	1.8	26.9	2.4	1.8	3.7
22	0	2.0	15.0	37.0	3.3	3.0	17.4	4.4	0	0	4.7
23	0	9.8	67.2	7.6	1.6	.2	47.1	34.9	6.4	0	1.5
24	0	2.1	20.1	60.0	.6	10.6	1.2	0	1.3	0	3.1
25	1.9	15.8	19.6	12.1	1.9	2.2	16.6	47.4	8.5	.6	5.2
26	1.3	2.2	10.9	12.8	.6	9.7	5.4	62.9	.5	.9	1.5
27	0	5.0	18.5	18.7	.3	0	8.0	8.7	6.2	0	8.2
28	0	4.8	24.4	8.6	2.1	5.9	32.3	30.7	9.4	0	2.3
29	0	0	21.6	22.9	.6	8.5	0	40.6	.4	0	0
30	0	6.2	15.9	16.1	1.3	3.2	0	9.7	0	0	20.0
31	0	0	20.2	38.2	2.6	5.3	0	32.3	.5	0	0
32	0	8.0	13.1	21.0	3.2	23.6	2.0	47.9	1.4	0	3.2
33	3.9	3.6	18.3	14.8	.8	1.8	9.8	69.3	6.2	.8	.8
34	0	1.2	21.5	10.9	1.0	.9	1.3	51.3	9.7	0	1.2
35	0	2.1	24.5	15.5	0	0	22.5	0	0	0	3.8
36	4.1	6.0	23.8	42.9	.6	13.2	4.6	0	1.5	6.3	9.2
37	0	3.3	19.2	8.3	0	2.0	39.9	21.3	0	0	4.3
38	.9	12.8	28.8	15.8	1.2	3.5	4.6	0	0	1.9	26.7
39	0	5.5	20.2	42.6	1.7	10.8	3.6	8.5	3.7	0	6.5
40	3.2	6.8	25.9	3.1	1.3	13.7	4.4	9.8	0	5.9	12.6
41	.9	3.2	27.8	27.5	1.5	2.5	2.7	4.7	0	1.6	5.8
42	1.1	10.3	46.3	43.1	2.1	3.3	18.4	4.6	.6	.6	5.3
43	0	3.5	25.2	25.6	2.3	12.9	26.5	0	0	0	5.6
44	0	20.1	18.7	31.0	2.2	3.9	31.3	61.4	1.2	0	29.1

Table iv. (continued)

	%sperm.transfer	g.antennate	g.turn	g.judder	g.groom	g.pause	g.chase	g.shake antennae	g.raise wings	time to mate	latency	courtship duration
1	25.2	19.8	1.2	1.0	11.8	475.8	0	40.4	0	213	177	98
2	6.7	48.9	3.6	9.0	8.7	524.1	11.9	7.6	0	819	580	239
3	9.7	269.6	12.8	6.1	0	257.0	36.8	5.5	0	211	21	190
4	77.7	141.2	12.5	1.5	6.1	378.7	25.0	27.2	0	48	22	26
5	58.2	0	17.9	0	54.3	6.6	4.7	20.2	0	150	122	28
6	27.1	191.6	1.8	6.5	34.5	343.8	4.6	34.1	0	261	135	126
7	25.3	181.2	12.7	22.2	0	333.6	32.6	7.7	0	296	241	55
8	4.6	38.6	11.9	8.8	8.7	494.6	27.0	1.5	0	904	506	398
9	18.5	218.5	3.0	5.1	16.6	313.5	.8	20.2	0	120	2	118
10	55.3	96.1	8.9	29.4	11.1	323.1	46.7	27.8	52.5	113	62	51
11	14.1	234.5	7.3	16.0	0	322.3	15.5	0	0	204	29	175
12	39.6	243.5	1.2	2.6	27.5	298.4	9.4	15.2	0	142	63	79
13	25.1	187.9	2.4	6.5	0	392.3	4.9	1.7	0	133	40	93
14	36.6	527.4	0	3.1	0	81.5	0	0	0	753	664	89
15	84.2	198.4	7.3	45.0	.7	316.4	35.2	29.7	0	234	208	26
16	32.8	145.8	4.3	36.8	0	395.0	16.4	9.8	0	161	115	46
17	60.6	235.4	.9	18.0	26.3	304.2	1.1	44.0	1.0	101	69	32
18	14.5	3.9	2.4	18.0	377.4	214.2	25.4	14.7	0	470	289	181
19	77.1	215.0	10.8	54.5	49.6	219.2	23.6	60.0	5.6	161	112	49
20	12.9	169.5	6.2	23.6	27.5	289.9	50.8	34.3	1.7	223	20	203

Table iv. (continued)

	%sperm.transfer	g.antennate	g.turn	g.judder	g.groom	g.pause	g.chase	g.shake antennae	g.raise wings	time to mate	latency	courtship duration
21	38.6	397.6	9.0	6.0	15.7	171.3	0	47.3	0	507	429	78
22	34.9	226.6	4.0	32.3	0	302.4	34.2	48.2	0	71	28	43
23	10.0	19.2	0	0	0	570.3	0	18.0	0	753	78	675
24	30.0	281.2	8.8	33.5	0	261.4	14.3	3.7	0	71	4	67
25	6.4	200.4	3.1	11.2	0	338.6	33.7	10.3	0	479	175	304
26	7.2	121.8	9.0	47.9	0	388.2	22.7	20.6	0	191	40	151
27	30.3	68.2	9.0	13.7	13.1	422.0	57.8	17.6	0	294	233	61
28	11.6	425.7	6.3	14.0	16.3	136.8	10.0	8.9	0	212	2	210
29	31.8	229.1	7.9	14.7	29.3	275.5	37.0	16.1	0	70	2	68
30	51.3	370.9	.8	1.6	22.9	190.9	4.3	11.9	0	231	201	31
31	27.7	119.8	10.9	7.9	33.5	348.7	75.3	11.6	0	90	17	73
32	5.2	263.4	9.0	42.5	20.5	216.9	64.0	43.6	0	379	126	253
33	3.9	351.6	8.6	18.8	33.3	153.3	23.8	64.0	.6	590	115	475
34	21.5	220.6	6.7	17.8	6.4	307.5	37.6	10.1	0	172	72	100
35	44.5	299.6	7.1	23.2	0	192.8	10.6	47.6	16.8	140	85	55
36	36.6	290.7	8.4	24.2	0	263.9	13.8	8.6	0	158	93	65
37	25.3	378.0	10.0	15.2	7.1	161.3	26.6	9.3	0	77	1	76
38	60.0	214.8	5.5	19.5	0	285.6	2.5	60.4	17.0	63	15	48
39	24.0	266.2	4.5	33.6	11.9	248.2	38.8	16.6	0	144	60	84
40	48.0	246.9	1.7	27.1	33.4	273.2	.5	65.0	0	244	190	54
41	50.5	546.9	.5	0	0	50.7	1.7	11.9	0	80	25	55
42	23.9	386.1	1.9	13.2	14.4	168.6	18.8	15.2	0	216	22	194
43	40.6	11.5	2.9	18.8	14.2	523.4	24.2	0	0	295	233	62
44	27.1	122.8	2.8	18.1	0	391.5	21.8	24.5	3.0	155	26	69

Appendix B

Table v. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the total duration of 9 courtship behaviour displays in Ad Libitum diet males, N=51. Significance level for sequential Bonferroni $p \leq 0.0056$ *.

Display	$\bar{x}$ ± s.d. (s)	Parasite F	Parasite p	Age F	Age p	Interaction F	Interaction p
antennate	26 ± 24	1.17	0.32	0.93	0.41	1.21	0.32
turn	2 ± 2	1.24	0.30	0.22	0.81	2.44	0.06
judder	10 ± 11	0.49	0.62	3.42	0.04	0.68	0.61
stridulate	18 ± 42	0.56	0.57	0.56	0.58	0.33	0.86
pause	108 ± 162	1.76	0.18	0.81	0.45	1.88	0.13
chase	8 ± 14	0.66	0.08	0.08	0.93	1.68	0.17
shake antennae	3 ± 5	2.75	0.08	2.59	0.09	1.11	0.36
raise wings	11 ± 14	0.16	0.85	3.31	0.05	0.92	0.46
spermatophore transfer	28 ± 8	0.20	0.82	0.35	0.71	0.37	0.83

Table vi. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the percent duration of courtship behaviour displays in Ad Libitum diet males, N=51. Significance level for sequential Bonferroni $p \leq 0.0056$ *.

Display	$\bar{x} \pm$ s.d. (%)	Parasite F	Parasite p	Age F	Age p	Interaction F	Interaction p
antennate	9 ± 6	0.63	0.54	2.47	0.10	0.28	0.89
turn	1 ± 1	0.42	0.66	1.06	0.36	0.93	0.46
judder	3 ± 3	0.55	0.58	7.36	0.003*	0.57	0.69
stridulate	4 ± 7	0.43	0.65	0.44	0.64	0.22	0.93
pause	21 ± 22	2.09	0.14	1.27	0.29	1.83	0.14
chase	2 ± 3	2.44	0.10	0.01	0.99	1.21	0.32
shake antennae	1 ± 3	1.96	0.15	4.54	0.02	0.74	0.57
raise wings	4 ± 4	0.21	0.81	2.89	0.07	0.37	0.83
spermatophore transfer	11 ± 8	1.49	0.24	0.59	0.57	0.98	0.43

Table vii. Results from Spearman Rank correlations on parasite load and percent duration of courtship behaviour displays in Nutritionally Deprived diet males, N=44. Significance level for sequential Bonferroni $p \leq 0.0056$ *.

Display	$\bar{x} \pm$ s.d. (%)	r_s	p
antennate	19 ± 13	-0.30	0.06
turn	1 ± 1	-0.06	0.69
judder	5 ± 5	-0.30	0.03
stridulate	13 ± 13	-0.006	0.97
pause	22 ± 21	0.08	0.61
chase	3 ± 8	-0.08	0.61
shake antennae	1 ± 2	0.02	0.90
raise wings	48 ± 263	-0.10	0.53
spermatophore transfer	32 ± 21	-0.005	0.97

Table viii. Results from 2-way ANOVAs on ranked data on the effect of parasite load and age on the total duration of 8 guarding behaviour displays in Ad Libitum diet males, N=51. Significance level for sequential Bonferroni $p \leq 0.0056$ *.

Guarding Display	$\bar{x}$ ± s.d. (s)	Parasite F	Parasite p	Age F	Age p	Interaction F	Interaction p
g.antennate	129 ± 120	0.45	0.64	0.22	0.81	0.74	0.57
g.turn	4 ± 3	2.07	0.14	5.33	0.01	2.54	0.05
g.judder	17 ± 15	0.01	0.99	0.94	0.40	1.43	0.24
g.groom	24 ± 27	0.14	0.87	0.40	0.68	0.73	0.58
g.pause	371 ± 114	0.58	0.56	0.42	0.66	0.57	0.69
g.chase	14 ± 22	0.02	0.98	0.60	0.55	0.19	0.94
g.shake antennae	27 ± 21	0.39	0.68	2.41	0.10	0.33	0.86
g.raise wings	2 ± 7	0.17	0.84	0.75	0.48	0.22	0.93

Appendix C

Figure i. The mean durations and standard deviations of the 9 courtship displays for Ad Libitum diet males, N=51.

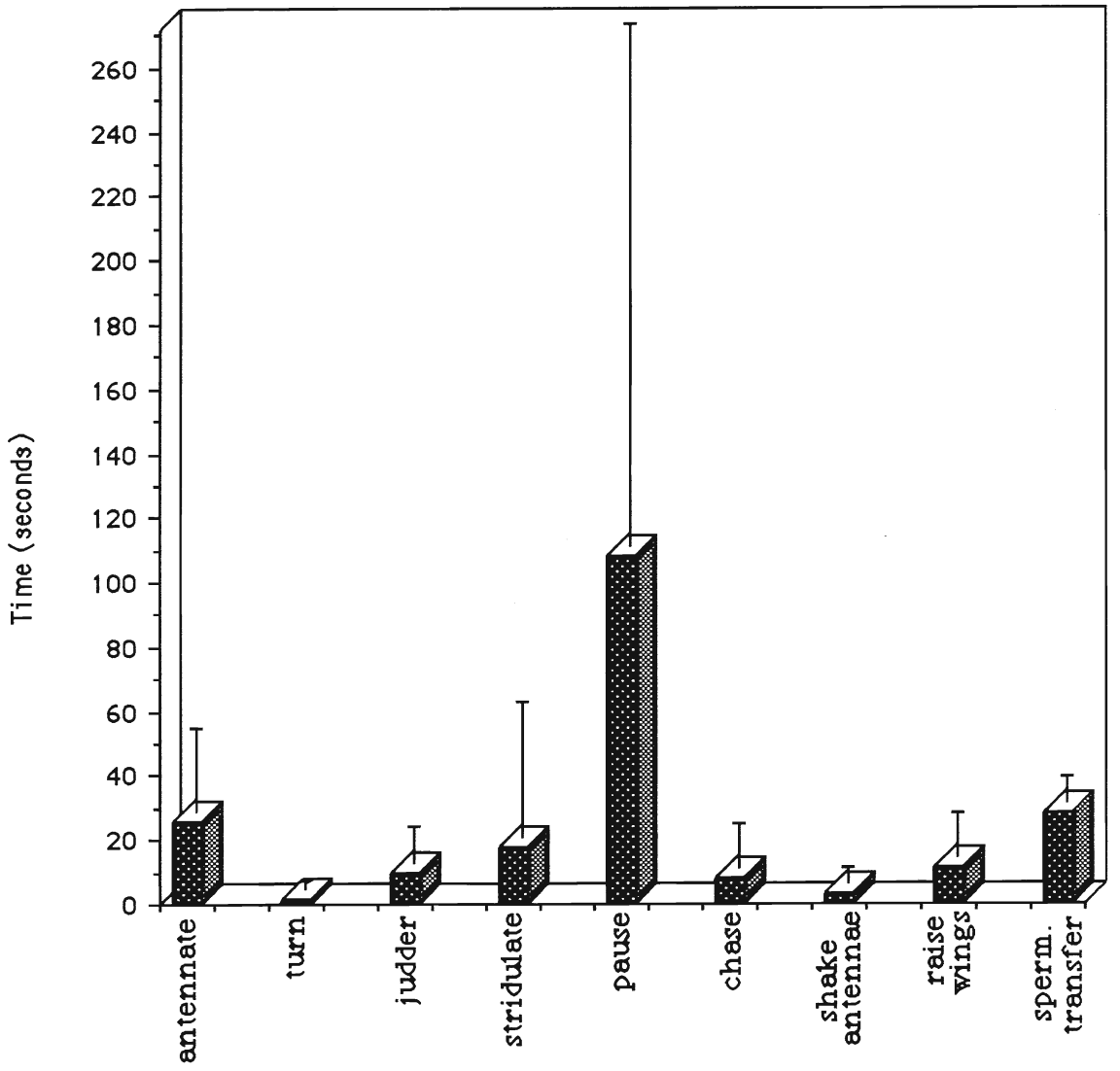

Time (seconds)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
antennate
turn
judder
stridulate
pause
chase
shake antennae
raise wings
sperm. transfer

Figure ii. The mean durations and standard deviations of the 9 courtship displays for Nutritionally Deprived diet males, N=44.

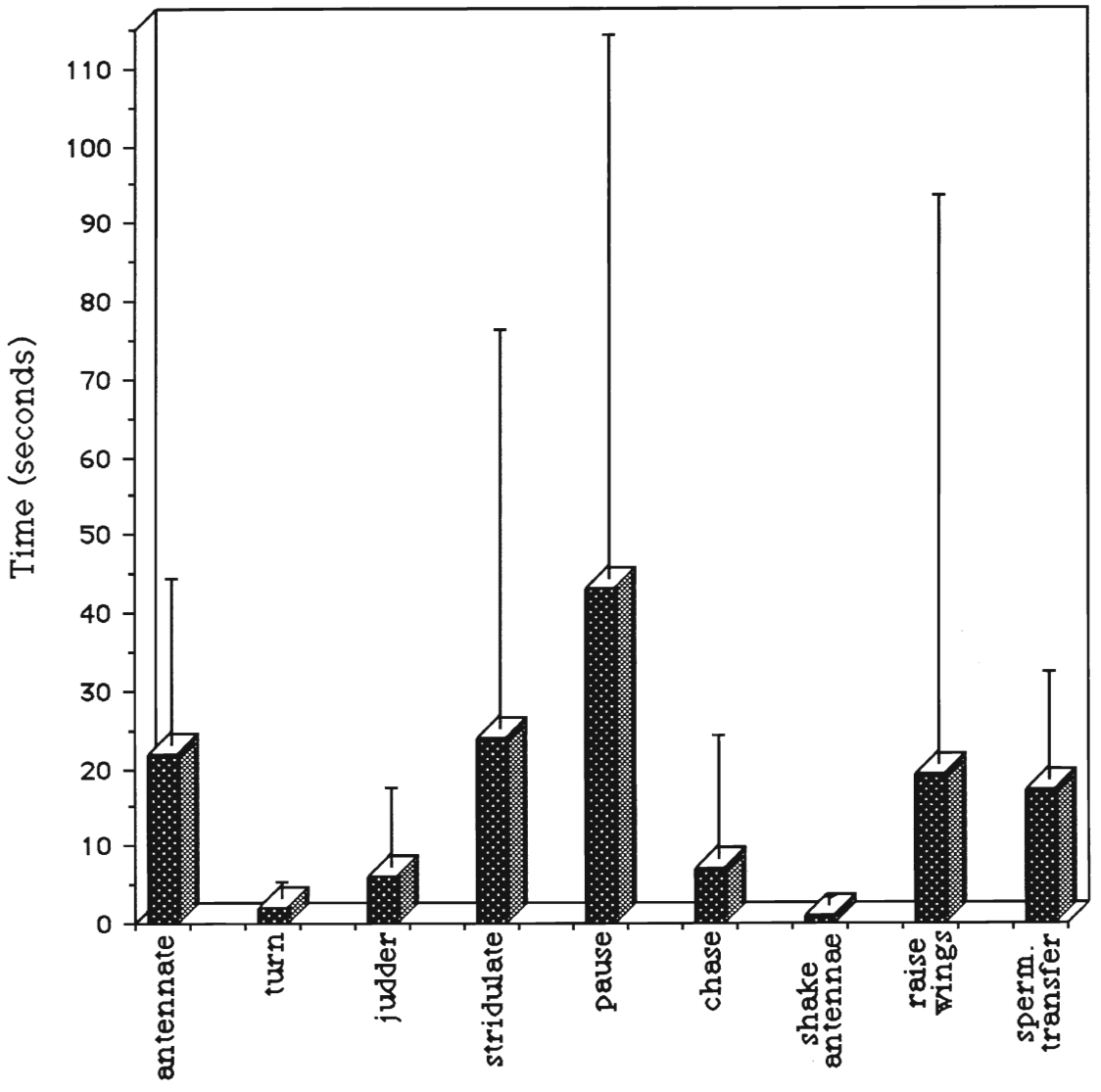

Time (seconds)
0
10
20
30
40
50
60
70
80
90
100
110
antennate
turn
judder
stridulate
pause
chase
shake antennae
raise wings
sperm. transfer

Figure iii. The mean proportions (% courtship time) and standard deviations of the 9 courtship displays for Ad Libitum diet males, N=51.

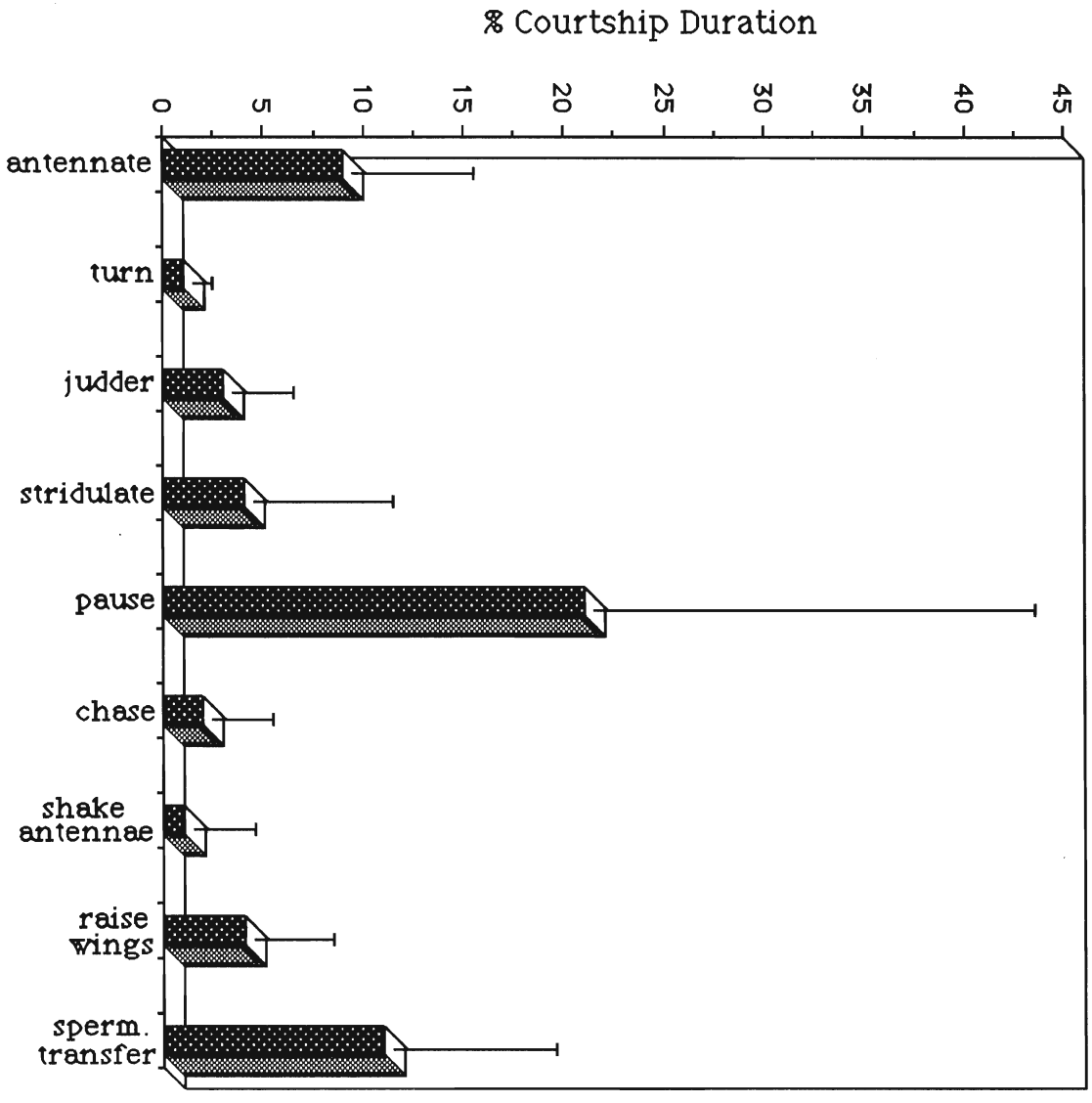

% Courtship Duration
0
5
10
15
20
25
30
35
40
45
antennate
turn
judder
stridulate
pause
chase
shake antennae
raise wings
sperm. transfer

Figure iv. The mean proportions (% courtship time) and standard deviations of the 9 courtship displays for Nutritionally Deprived diet males, N=44.

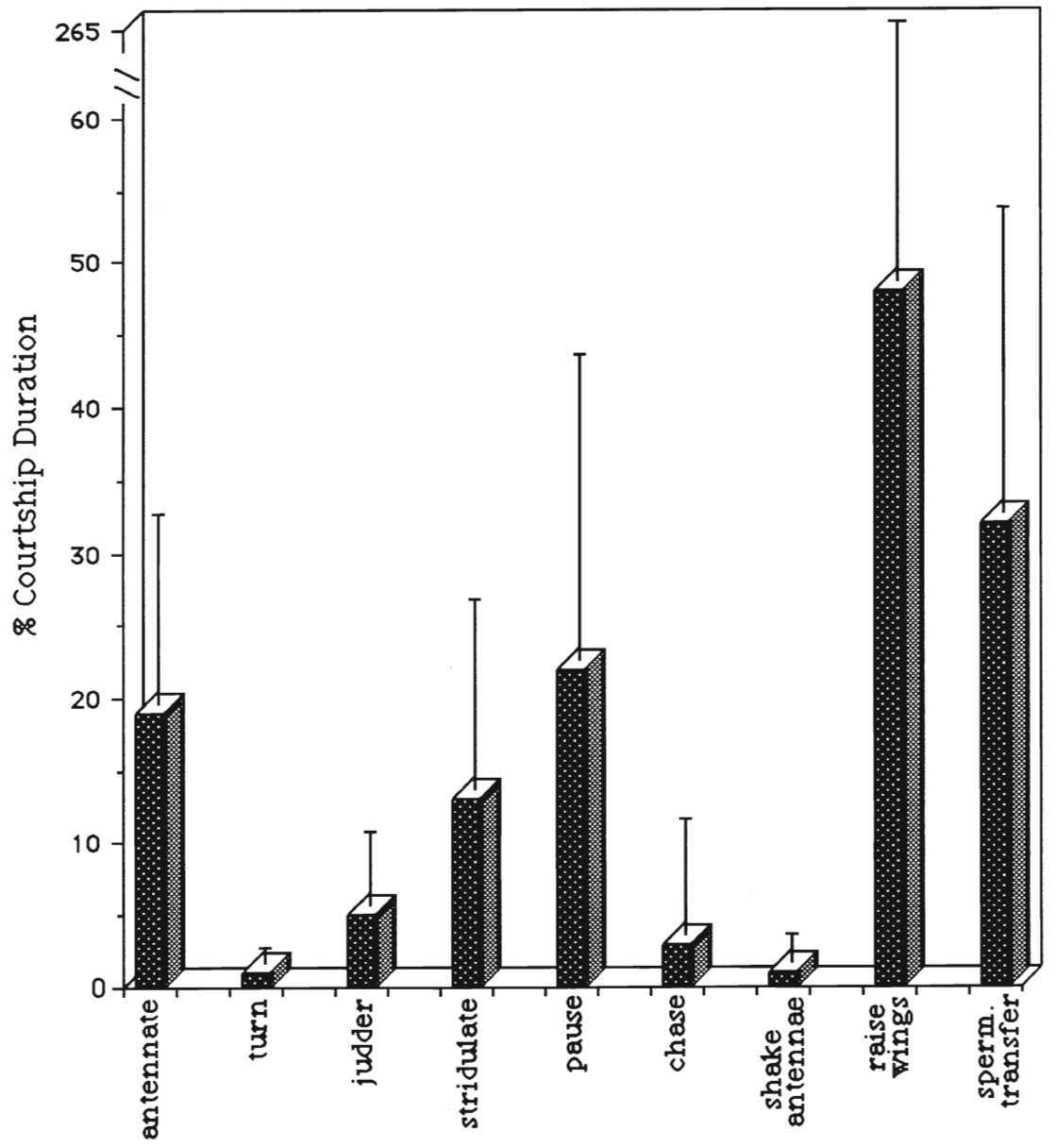

% Courtship Duration
265
60
50
40
30
20
10
0
antennate
turn
judder
stridulate
pause
chase
shake antennae
raise wings
sperm. transfer

Figure v. The mean durations and standard deviations of the 8 guarding displays for Ad Libitum diet males, N=51.

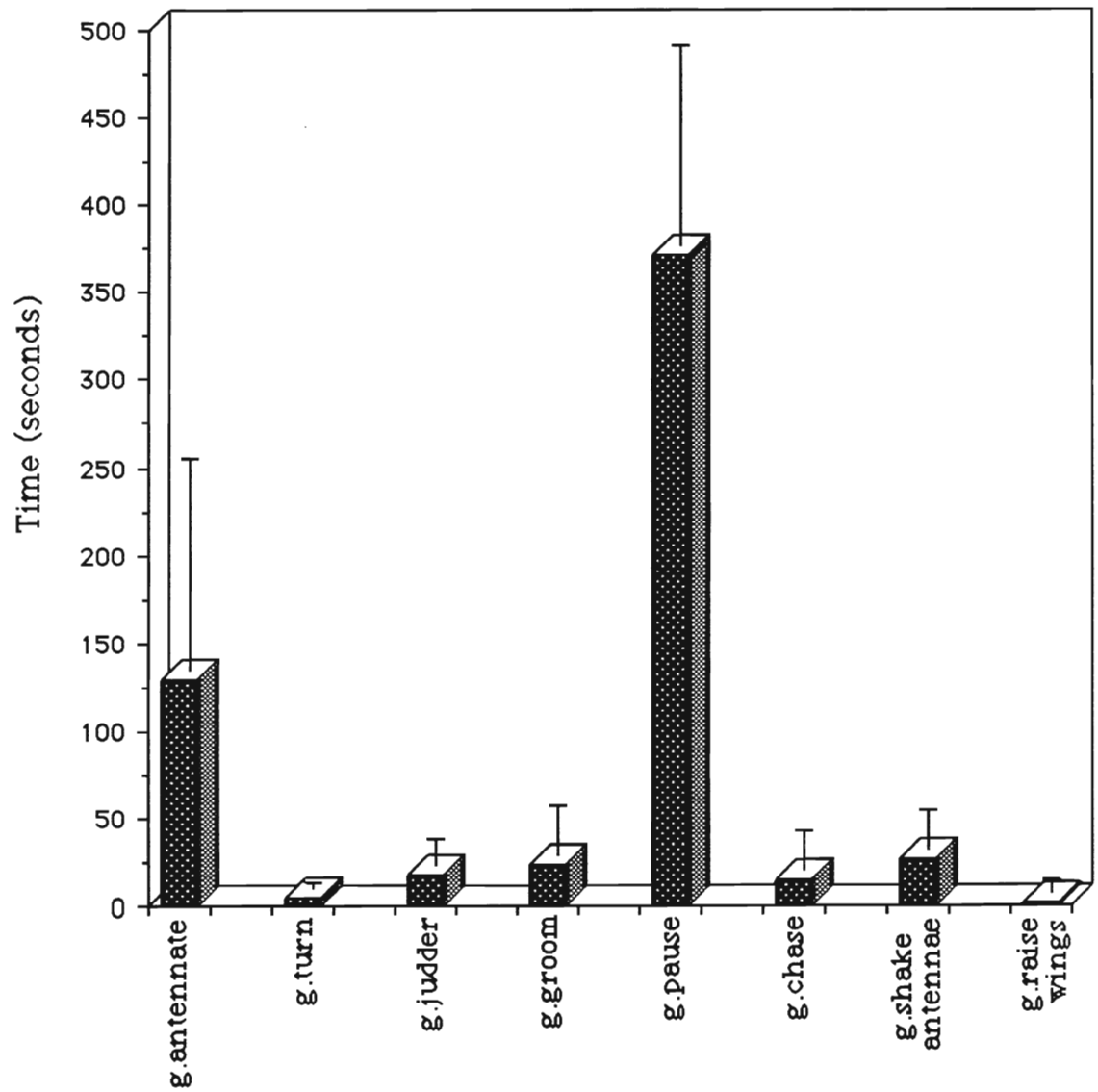
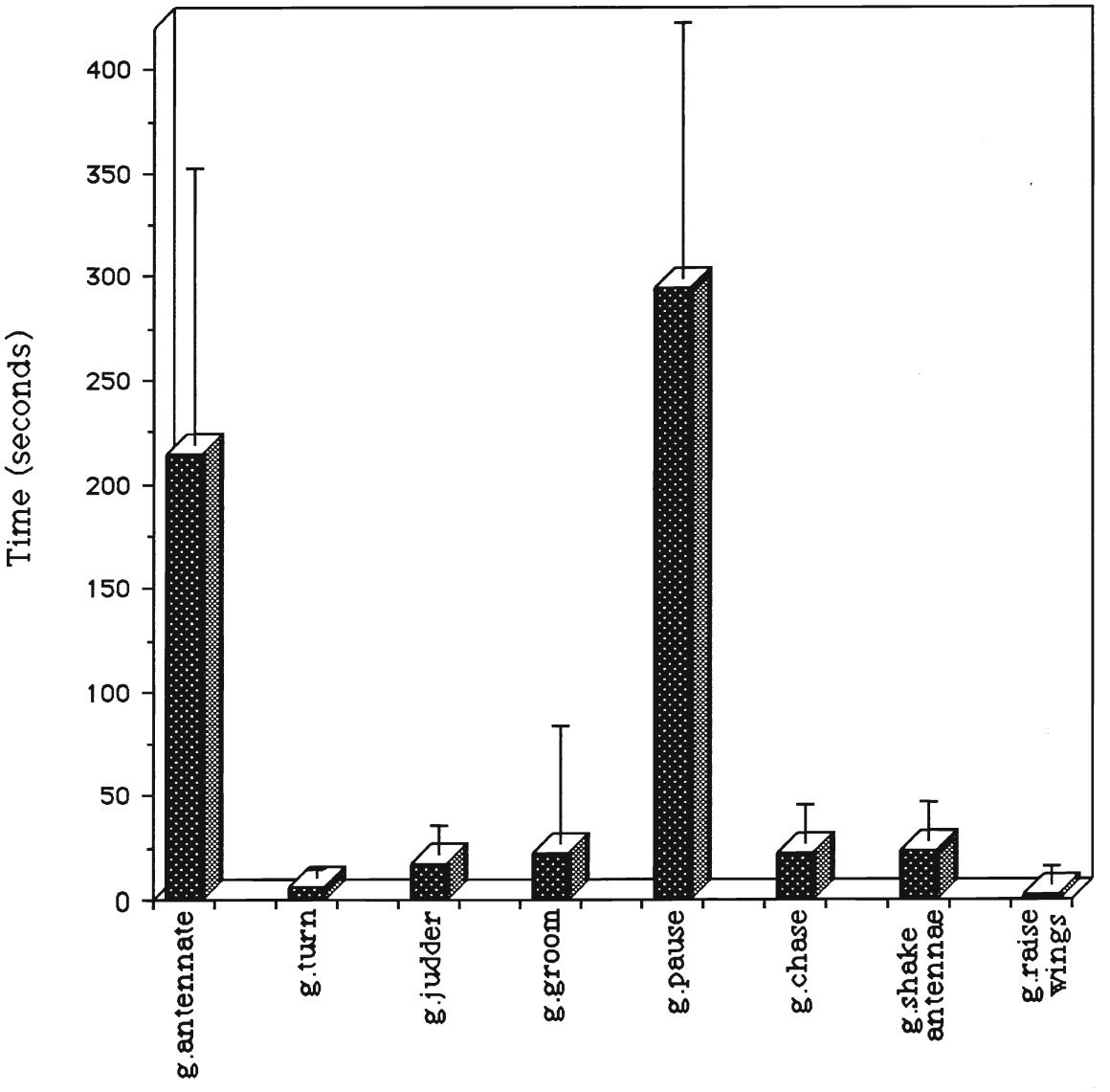

Time (seconds)
500
450
400
350
300
250
200
150
100
50
0
g.antennate
g.turn
g.judder
g.groom
g.pause
g.chase
g.shake antennae
g.raise wings

Figure vi. The mean durations and standard deviations of the 8 guarding displays for Nutritionally Deprived diet males, N=44.


Time (seconds)
400
350
300
250
200
150
100
50
0
g.antennate
g.turn
g.judder
g.groom
g.pause
g.chase
g.shake antennae
g.raise wings